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Drinking Water Criteria Document on Nitrate/Nitrite

Life Systems, Inc., Cleveland, OH

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16. ABSTRACT

This document deals with the drinking water health effects of Nitrate/Nitrite through the review of several studies. These studies include animal and humans. Physical and chemical properties are discussed. Carcinogenicity of the compound is reviewed and evaluated through various studies. Any existing guidelines and standards are presented.

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgmenc of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is non-enforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLGs for nitrate and nitrite. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity were evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document was comprehensive, only the reports considered most pertinent in the derivation of the MCLGs are cited in the document. The comprehensive literature data base in support of this document includes information published up to May, 1990; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (One-day, Ten-day and Longer-term, approximately 10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLGs, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

Michael B. Cook
Director
Office of Drinking Water

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I. SUMMARY

Nitrate (NO_3^-) is a relatively stable component of the natural environment. It exists mainly in combination with alkali metal cations such as Na^+, K^+ or Ca^{+2}, all of which are readily soluble in water. The main use for nitrate is as a fertilizer, but is also used in explosives and glassmaking, and as a food preservative.

Nitrite (NO_2^-) is produced by chemical or bacterial reduction of nitrate. It is used mainly as a food preservative. Nitrite is less stable than nitrate and many undergo a variety of reactions. With respect to health effects, the most important of these reactions are: 1) oxidation of other substances (e.g., Fe^{+2} in hemoglobin to Fe^{+3} in methemoglobin), and 2) reaction with secondary and tertiary amines to form nitrosamines.

Toxicokinetics

The primary route of nitrate and nitrite absorption is through the gastrointestinal tract. Nitrate is absorbed by active transport through the wall of the upper small intestine. Nitrite is absorbed by diffusion across the gastric mucosa and also through the wall of the intestinal tract. In both cases, absorption following ingestion is essentially complete (i.e., 100%). Nitrate and nitrite can also be absorbed into the blood through the lungs.

Nitrate is rapidly disseminated throughout the body in the blood and is actively secreted in saliva, gastric juices, and urine. Nitrite reacts so rapidly with hemoglobin that little is transported to the tissues. Nitrate and nitrite do not bioaccumulate. However, they are constantly present in the body as a result of de novo synthesis as well as ingestion.

Nitrate is reduced to nitrite by the action of oral and gastrointestinal bacteria. In healthy adults and older children, approximately 5% of an ingested dose of nitrate is converted to nitrite. This occurs mainly in the mouth, due to the action of oral bacteria on nitrate secreted in the saliva. In infants, nitrate may also be reduced to nitrite in the stomach. This is because the infant stomach may have a pH sufficiently high to permit bacterial growth. No quantitative data are available on the amount of nitrate that is reduced in the alimentary tract (mouth, stomach) of susceptible infants, but it is generally believed to be considerably higher than in adults. Nitrite that is absorbed into the blood reacts with hemoglobin, oxidizing it to methemoglobin. Nitrite in the stomach may react with amines and other nitrogenous substrates present in food or in the epithelium to form N-nitroso compounds.

Nitrate is excreted readily through the kidneys. When excess nitrate is not present to the diet, both rats and humans excrete significantly more nitrate than they ingest, revealing the importance of endogenous formation of nitrate in vivo. Nitrite that is ingested or formed in vivo is so reactive that none remains to be excreted.

Health Effects in Animals

The toxic effects of nitrate are closely related to its conversion to nitrite by bacteria in the alimentary tract. Thus, the toxicity of nitrate depends not only on dose, but also on the level and type of bacteria present. Consequently, dose-response relationships are quite variable between different animal species. For example, ruminants are highly sensitive, while dogs have low sensitivity. Because of this species variability, animal data should be extrapolated to humans with caution.

The principal health effect from exposure to nitrate or nitrite is methemoglobinemia. This results when nitrite oxidizes the Fe^{+2} form of iron in hemoglobin to the Fe^{+3} state. In rats, acute oral LD_{50} values for $NaNO_3$ range from 1,000 to 2,000 mg/kg. These are close to LD_{50} values for NaCl, suggesting that acute lethality from $NaNO_3$ is caused mainly by nonspecific electrolyte imbalance. Chronic exposure to doses up to 69 mg nitrate-nitrogen/kg/day did not produce methemoglobinemia in rats, but doses of 50 mg/kg/day or higher have been noted to produce mild histological changes in liver or spleen. High doses may also interfere with thyroid function.

Nitrite is substantially more toxic to rodents than nitrate. Acute oral LD_{50} values range from 40 to 120 mg/kg (20-40 mg nitrite-nitrogen/kg/day), while doses of 20 mg nitrite-nitrogen/kg/day can produce methemoglobinemia and other related histological effects. High doses of nitrite act on vascular smooth muscle to produce vasodilation and hypotension, and may also cause changes in coronary blood vessels.

Numerous studies on reproductive function, teratogenicity and fetal development in several animal species have not revealed evidence of adverse effects from nitrate or nitrite except at relatively high dose levels. Such effects are likely to be secondary to maternal or fetal methemoglobinemia and related toxicity. Exposure of males to high doses may result in cytogenetic abnormalities in spermatocytes and morphological abnormalities in sperm, but this did not result in any apparent effect on reproduction or offspring. Ingestion of 20-100 mg nitrite-nitrogen/kg/day by dams during lactation can cause iron deficiency in milk and moderate to severe anemia and other post-natal effects in sucklings.

Most investigations on the mutagenicity of nitrate and nitrite have been negative. However, many studies have reported that both nitrate and nitrite lead to a variety of chromosomal aberrations, both in cultured cells _in vitro_ and in animals exposed _in vivo_.

The carcinogenic potential of nitrate has not been extensively studied in animals, but several investigations have not revealed any evidence of increased tumor incidence. The carcinogenic potential of nitrite has been much more thoroughly studied. One early study was interpreted as providing evidence of increased tumors of the lymphoreticular system in rats, but re-evaluation of the histological slides from this study led to the conclusion that the lesions were not neoplastic. Numerous other studies of nitrite carcinogenicity in animals have also been negative. However, when nitrite is given to animals along with high levels of nitrosable amines, clear increases in tumor incidence are often observed in a variety of tissues. This tumorigenic response is probably a result of the formation of carcinogenic nitrosamines by reaction of nitrite with the nitrosable substrates in the stomach.

Health Effects in Humans

The principal adverse health effect associated with exposure of humans to nitrate is methemoglobinemia in infants (age 0-3 months). This group is much more susceptible than older children and adults because the pH of the infant stomach can often be sufficiently high (above pH 4) to permit growth of bacteria that reduce nitrate to nitrite. Infants also ingest more water per unit body weight (about 0.16 L/kg/day) than older children (about 0.1 L/kg/day) or adults (about 0.029 L/kg/day).

Low levels of methemoglobin occur in normal individuals, with typical values usually ranging from 0.5% to 2.0%. However, due to the large excess capacity of blood to carry oxygen, levels of methemoglobin up to around 10% are not associated with any significant clinical signs. Concentrations above 10% may cause a bluish-color to skin and lips (cyanosis), while values above 25% lead to weakness, rapid pulse and tachypnea. Death may occur if methemoglobin values exceed 50%-60%. Many studies have been performed on the levels of nitrate in water that lead to clinically significant methemoglobinemia in infants. These studies have shown that most cases are associated with exposure to water containing 20 mg nitrate-nitrogen/L or higher, and that no significant effects occur if water contains 10 mg nitrate-nitrogen/L or less. In adults, methemoglobinemia has only been reported following accidental ingestion of large quantities of sodium nitrite (usually mistaken for table salt).

Only two studies are available on possible developmental or reproductive effects of nitrate in humans. One study suggested that there might be an increased risk of birth defects associated with ingestion of water containing 5-15 mg nitrate-nitrogen/L, but there were a number of other factors that could also have been responsible for the observation. No significant relation between nitrate levels in water and birth defects was detected in the second study.

Many epidemiological studies have been performed to determine if ingestion of nitrate or nitrite are associated with increased cancer risk. Some of these studies have noted increased cancer risk (especially gastrointestinal cancer) in populations ingesting above average levels of nitrate or nitrite in water or food. However, these studies are not adequate to show that nitrate/nitrite caused the increased risk, since reliable dose-response data are usually lacking, and there are many other risk factors for gastrointestinal cancer that might have been responsible.

Quantification of Toxicological Effects

Clinically significant signs of methemoglobinemia are not observed in infants exposed to drinking water containing 10 mg nitrate-nitrogen/L or lower. This value is supported by many epidemiological studies and case reports from the United States and elsewhere. This concentration corresponds to an average daily intake of 1.6 mg nitrate-nitrogen/kg/day by an infant, and this is taken to be the Reference Dose (RfD) for nitrate for an infant. This value is also protective for older children and adults, since infants are the most sensitive subpopulation.

Data are not available to identify the NOAEL for nitrite in infants. Based on the assumption that infants convert at least 10% of ingested nitrate to nitrite, the NOAEL is estimated to be at least 1 mg nitrite-nitrogen/L. Based on this, the RfD for nitrite is 0.16 mg nitrite-nitrogen/kg/day for an infant. This value is also protective for older children and adults, since they are somewhat less susceptible to nitrite-induced methemoglobinemia than infants.

The USEPA is currently evaluating the evidence that ingestion of nitrate or nitrite may be associated with increased risk of cancer. Cancer weight-of-evidence categories have not yet been assigned.

II. PHYSICAL AND CHEMICAL PROPERTIES

A. Physical and Chemical Properties

Some important physical and chemical properties of nitrate and nitrite are summarized in Table II-1. Nitrate ion (NO_3^-) is the physicochemically stable form of combined nitrogen for oxygenated aqueous systems. The nitrate salts of all common metals are quite soluble in water. Nitrate has little tendency to form coordination complexes with metal ions in dilute aqueous solution. In dilute aqueous solutions, nitrate is chemically unreactive, and nearly all of the transformations involving nitrate in natural waters are mediated biochemically (NAS 1978).

Nitrite ion (NO_2^-) contains nitrogen in an intermediate and relatively unstable oxidation state. Both chemical and biological processes can result in the further reduction of nitrite to more stable compounds or in oxidation back to nitrate. Enzymatic processes in certain bacterial, fungal and plant systems can reduce nitrate and both oxidize and reduce nitrite as part of the natural nitrogen cycle (NAS 1978).

Nitrite ion is the conjugate base of a weak acid, nitrous acid (HNO_2). In most natural waters, the acid is dissociated into ions, but in acidic environments, a significant fraction can exist as the undissociated species. Of the many reactions involving nitrous acid, the reaction with amino

Table II-1 Properties of Nitrate and Nitrite

	Nitrate	Nitrite
Formula	NO_3^-	NO_2^-
Usual state	Ion in aqueous solution at neutral pH	Ion in aqueous solution at neutral pH
Acid	Conjugate base of strong acid HNO_3, pKa = -1.3	Conjugate base of weak acid HNO_2, pKa = 3.4
Salts	Nearly all soluble in water, dissociated at neutral pH	Forms salts with Li Na K Ca Sr Ba Ag
Reactivity	Unreactive	Reactive Oxidizes antioxidants Oxidizes Fe^{++} of hemoglobin to Fe^{+++} Oxidizes primary amines Nitrosates secondary amines, tertiary amines, ureas, carbamates, guanidines

Adapted from NAS 1978, 1981

substrates to form N-nitroso compounds is of special concern because many N-nitroso compounds are carcinogenic (NAS 1978).

Nitrosation in aqueous nitrous acid at pH<5 proceeds by reaction of the unprotonated amino substrate with an electrophilic nitrosating agent such as dinitrogen trioxide (N_2O_3) or the hydrated nitrosonium ion (H_2O^+-NO). Basic secondary amines react most rapidly at pH 3.4, but these reactions are generally slower than those for amides, ureas and carbamates (NAS 1981).

Nitrosation of secondary amines by aqueous nitrous acid is accelerated by nucleophilic anions such as thiocyanate and iodide. Comparable nitrosation reactions of amides, ureas, guanidines and carbamates are not usually accelerated by these catalysts (NAS 1981).

Nitrosation of secondary amines, amides, ureas and guanidines by aqueous nitrous acid can be inhibited by compounds that reduce nitrous acid to nitric oxide. These inhibitors include ascorbic acid (vitamin C), α-tocopherol (vitamin E) and several naturally occurring polyphenolic antioxidants (NAS 1981).

B. Manufacture and Uses

The major use of nitrate is in inorganic fertilizers. A production summary of the principal inorganic nitrogen fertilizers for August 1981, as reported by the U.S. Bureau of the Census, in short tons (2,000 lbs) is as follows: ammonia - 1,510,000; ammonium nitrate - 617,000; nitric acid - 657,000 (USDC 1981). Other uses include the production of explosives and the production of purified potassium nitrate for glassmaking.

Sodium nitrite is produced for use as a preservative in cured meats. Nearly four billion kilograms of cured meat products were processed with added nitrite in the United States during 1979. Nitrate is also added to a variety of products to serve as a source of nitrite by bacterial reduction of nitrate to nitrite. This serves to inhibit further bacterial growth (NAS 1981).

C. Summary

Nitrate (NO_3^-) is a relatively stable component of the natural environment. It exists mainly in combination with alkali metal cations such as Na^+, K^+ or Ca^{+2}, all of which are readily soluble in water. The main use for nitrate is as a fertilizer, but is also used in explosives and glassmaking, and as a food preservative.

Nitrite (NO_2^-) is produced by chemical or bacterial reduction of nitrate. It is used mainly as a food preservative. Nitrite is less stable than nitrate and many undergo a variety of reactions. With respect to health effects, the most important of these reactions are: 1) oxidation of other substances (e.g., Fe^{+2} in hemoglobin to Fe^{+3} in methemoglobin), and 2) reaction with secondary and tertiary amines to form potentially carcinogenic nitrosamines.

III. TOXICOKINETICS

A. Absorption

Witter et al. (1979b) administered oral radioactive nitrate ion ($^{13}NO_3^-$) (dose not reported) to two male volunteers and followed the radioactive label with a computer-linked Auger camera. One subject received nitrate orally about an hour after a large meal. The half-time for the disappearance of the label from the stomach was about 30 minutes. The radioactivity in the pylorus remained almost constant, suggesting that nitrate is not rapidly absorbed from the stomach but exits into the small intestine. The second subject was given nitrate about ten hours after eating. The half-time for the disappearance of the label from the stomach of the second subject was less than ten minutes.

A similar study was performed (doses not reported) with Sprague-Dawley rats of undisclosed sex, some of which were previously ligated at the pyloric valve (Witter et al. 1979a). The group with pyloric ligation retained nearly all of the radioactivity in the stomach 40 to 50 minutes after administration. Only 21% of the radioactivity was retained in the rats without pyloric ligation, suggesting that nitrate leaves the rat stomach predominantly through the pyloric valve and is not absorbed from the stomach. In the rats which were not ligated, essentially all of the radioactivity passed into the upper small intestine, where most was absorbed. Similar studies using $^{13}NO_2^-$ resulted in some gastric absorption, but most of the radioactivity seemed to exit the stomach via passage into the duodenum. The exit of nitrite from the stomach was slower, and less radioactivity appeared to be absorbed from the small intestine than with nitrate.

Witter and Balish (1979) studied oral absorption of nitrate and nitrite ions using a spectrophotometric assay to differentiate the ions from their metabolites. Sprague-Dawley rats (60- to 90-days-old, sex not reported) were exposed separately to both nitrate and nitrite in drinking water at 1 mg/mL (160 mg/kg/day; equivalent to 36.2 and 48 mg/kg/day of nitrate- and nitrite-nitrogen, respectively) for an unreported period. Various parts of the intestinal tract were removed and analyzed for nitrate and nitrite ion content. Rats fed nitrate had both nitrate and nitrite in the stomach, but only nitrate in the small intestine. Rats fed nitrite had both ions in the stomach and small intestine. Control rats had trace amounts of only nitrate in their stomachs. These results are in agreement with the above results (Witter et al. 1979a, 1979b), suggesting that nitrate is absorbed from the upper small intestine and nitrite from the stomach. However, when a large dose of nitrite is administered, some passes into the intestine.

Friedman et al. (1972) performed a colorimetric assay of nitrite absorption in male Swiss ICR/Ha mice weighing between 20 and 25 grams. Mice were given food and water ad lib while 150 µg of sodium nitrite (6.8 mg/kg; 1.4 mg/kg as nitrogen) was administered to each mouse by gavage. Groups of 13-18 mice were sacrificed within 1 minute and at 10, 20 and 30 minutes after sodium nitrite administration, their stomachs were removed and assayed. At ten minutes after oral administration, 85% of the administered sodium nitrite had disappeared from the mouse stomach. Ligation of the gastroduodenal junction had no effect on nitrite absorption. Other experiments were performed using isolated mouse stomachs where absorption of nitrate was prevented. After a 30-minute incubation, there was 63% loss of nitrite, of which 40% had been converted to nitrate. The authors concluded that the major pathway for disappearance of available gastric nitrite is absorption directly from the stomach into the bloodstream. The rate of disappearance of nitrite from the mouse stomach fits second-order kinetics.

Parks et al. (1981) studied the absorption of $^{13}NO_3^-$ and $^{13}NO_2^-$ after intratracheal inscillation of 10-100 ng/kg (0.00001 to 0.0001 mg/kg) in groups of 10-12 Balb/C mice. In this study 100% of the label was absorbed within 10 minutes.

No information on dermal absorption of nitrate or nitrite was found in the literature.

B. Tissue Distribution

Witter et al. (1979b) gavaged nine rats (sex and strain not reported) with $^{13}NO_3^-$ (dose not reported) and 45-60 minutes later organs were removed and the radioactivity measured. The largest fraction, 47.3%, was found in the carcass after removal of abdominal organs. This was more than could be accounted for as blood-bound activity. To study the fate of blood nitrate, the same authors injected $^{13}NO_3^-$ intravenously into nine rats of which one was ligated at the pyloric junction and three at the ileocecal junction. Except for the stomach, which contained 5.6%-6.8% of the radioactivity, no marked accumulation was seen in any organ at 50 minutes and the radioactivity was mainly in the eviscerated carcass (74.2%-78.8%). Thirty minutes after injection, the intestinal tract from the duodenum to the large intestine contained essentially the same amount of radioactivity even if the pylorus was ligated. This indicated that the label of $^{13}NO_3^-$ could enter the intestines from the blood, either as part of the biliary or pancreatic secretions or by direct secretion from the bloodstream. Data from rats ligated at the ileocecal junction suggested secretion directly into the lower intestines from the blood.

Witter et al. (1979b) also injected $^{13}NO_3^-$ intravenously into a human volunteer and followed the radioactivity with an Auger camera. There was rapid distribution of the label. The radioactivity in the heart region reached a maximum of about 3% of the administered dose at 2 minutes, as it passed through the heart for the first time. The activity then fell rapidly in the next 2 minutes as the radioactive label became evenly distributed in the blood. The radioactivity accumulated almost linearly with time in a fist-sized region of the abdomen, probably due to the swallowing of salivary $^{13}NO_3^-$. The remainder was fairly evenly distributed throughout the body, with less than 5% measured in the urine or salivary glands within 40 minutes.

Parks et al. (1981) studied the distribution of $^{13}NO_3^-$ and $^{13}NO_2^-$ after intratracheal instillation in Balb/C mice and intravenous injection in mice and New Zealand White rabbits. Mice were sacrificed from 5 to 30 minutes after administration of the labeled ions and organs were excised and weighed. Tracer concentrations were determined by gamma-ray counting. Both tracers achieved transient steady-state very rapidly for each route of administration. The distribution data were relatively constant within 5 minutes. The specific activities did not differ significantly between organs or routes of administration. The authors concluded that either the ions distribute equally into the same body space or they chemically transform into each other or into similar products.

The rabbits injected intravenously were studied with the Auger scintillation camera and by blood analysis. A rapid homogeneous distribution of the radioactivity throughout the rabbit was observed. Steady-state between the intravascular and extravascular compartments was reached within 5 minutes after injection of either radiochemical. Only 2%-3% of the label appeared in the bladder during the first 30 to 45 minutes after injection (Parks et al. 1981).

Portions of each supernatant from the blood distribution experiments in mice and rabbits were analyzed by high-pressure liquid chromatography (HPLC). The chromatographic results obtained from mouse plasma taken 10 minutes after intratracheal instillation of labeled nitrite revealed that 70% had been converted to nitrate, 27% remained nitrite and about 3% was in nonionic compounds. The product distribution in rabbit plasma 10 minutes after intravenous injection of labeled nitrite was 51% nitrate, 46% nitrite and 3% in nonanionic compounds. Ten minutes after administering labeled nitrate to both species, the label found in blood remained nitrate. Any nitrite formed by the bacterial reduction of nitrate was not detectable (Parks et al. 1981).

Edwards et al. (1954) studied the secretion of ionic compounds in normal people and patients undergoing treatment with radio-iodine ($^{131}I^-$). Numbers of subjects and doses employed were not reported. Anions were given as oral doses of the potassium salt and thiocyanate was detected by chemical assays.

They concluded that there is antagonism between iodide, thiocyanate, perchlorate, and nitrate for salivary secretion, thus suggesting a common transport mechanism for these ions. Nitrate was less effective than thiocyanate or perchlorate in depressing salivary secretion of iodide, and less effective than iodide or perchlorate in depressing salivary secretion of thiocyanate.

Spiegelhalder et al. (1976) used a colorimetric assay to study secretion of nitrate in saliva of 11 volunteers after ingestion of vegetables and vegetable juices. Nitrate concentration was determined at intervals of 30 or 60 minutes for 7 hours. The increase in the amount of nitrate secreted by the salivary glands was found to depend directly on the amount of nitrate ingested above a threshold dose of 54 mg. About 20%-25% of ingested nitrate is secreted. The concentration rises 30 minutes after nitrate ingestion and peaks from 1 to 3 hours later depending on the food or juice consumed. Swallowed salivary nitrate recirculates through the blood to the saliva again, causing one or more additional smaller peaks of secretion.

Secretion of nitrate by the gastric mucosa was studied by Bloomfield et al. (1962) in pylorus-ligated rats of an unreported strain. Doses of sodium nitrate ranging from 60 to 200 mg/kg (equivalent to 9.9-33 mg/kg as nitrogen) were injected intraperitoneally into fasted rats. As the plasma concentration increased, the gastric juice concentration also increased. The gastric juice to plasma ratio dropped from a value of 20 when the nitrate concentration in plasma was 1 mM to unity at 4.5 mM. This suggests that an active transport mechanism is involved and it can be saturated. When nitrate and $^{131}I^-$ were injected simultaneously, there was less radioactivity in the gastric juice than when $^{131}I^-$ was injected alone. This suggests that nitrate and iodide have a common concentrating mechanism in the rat stomach.

C. Metabolism

1. Reduction of Nitrate in Saliva

Nitrate can be reduced to nitrite and then to ammonia and other products by bacteria. The reduction of nitrate in saliva by human oral bacteria was studied by Goaz and Biswell (1961). Saliva was obtained by paraffin stimulation, collected in test tubes immersed in crushed ice, and used in less than an hour. Nitrate and nitrite concentrations were determined colorimetrically. When whole saliva containing 28 mg/L nitrate (6.4 mg nitrate-nitrogen/L) was incubated at 37°C, nitrate concentration decreased rapidly and was not detectable after 75 minutes. Nitrite accumulated transiently, increasing to 5 mg/L (1.5 mg nitrite-nitrogen/L) at 45 minutes and then decreasing to zero by 105 minutes of incubation. Saliva depleted of its endogenous nitrate and nitrite by prior incubation will rapidly reduce added nitrate. Further experiments determined that nitrate reduction to nitrite in human saliva occurs under either aerobic or anaerobic conditions, but the presence of oxygen completely inhibits the reduction of nitrite. Maximum activity occurred between pH 6 and 6.4, with no reduction of nitrate below pH 4 or above pH 9. Between pH 8.2 and 8.6 nitrate is reduced to nitrite but nitrite reduction is inhibited.

Eisenbrand et al. (1980) studied salivary nitrate reduction using 11 volunteers who had ingested varying amounts of nitrate in red beet juice or spinach. Using a colorimetric assay, they showed a direct correlation between the amounts of nitrite produced in saliva and the amounts of nitrate ingested. About 20% of the secreted nitrate was converted to nitrite. The same authors determined the mean and range of nitrite concentrations in saliva of different age groups in Germany. There was a steady increase from a mean of 0.6 mg/L at 1 week to 6 months of age, up to a mean of 6.8 mg/L in adults.

2. Reduction of Nitrate in the Stomach

Franklin and Skoryna (1971) studied the survival of microorganisms in the gastric lumen of fasting human subjects. The subjects were 13 hospitalized adults having sterilized nasogastric tubes in place. Sterile samples of gastric fluid were taken hourly and cultured. It was concluded that the survival of bacteria in the gastric lumen depends on the pH of the gastric juice. At pH 4 or higher, all samples showed growth of microorganisms and the percent of samples with few or no organisms increased with decreasing pH. When a rapid drop in pH occurred in a patient previously measured at pH greater than 4, the clearing of viable organisms occurred within 1 hour in most cases. The pH of a normal adult human stomach is less than 3.

Lactobacilli in breast-fed infants do not reduce nitrate to nitrite and maintain a pH low enough to discourage other bacteria from colonizing the infant's stomach. Marriott and Davidson (1923) determined the normal acidity of infants' stomachs (under 1-year-old) using four-color pH indicators to assay samples of gastric contents. Samples were withdrawn through a rubber catheter 2 hours after feeding different diets. Three mL of gastric contents were dialyzed against 3 mL of sterile physiologic saline for 15 minutes. The dialysate was assayed for pH and compared with the standard buffer solution. The gastric pH of 23 breast-fed normal infants averaged 3.75. When these infants were fed undiluted cow's milk, which has a high buffering capacity, their average gastric pH rose to 5.3. This is high enough to allow heavy bacterial growth. In another group of infants accustomed to cow's milk, the average pH following a meal of undiluted whole cow's milk was 4.75, indicating an adaptation to this type of food. A group of 16 infants aged 3 weeks to 9 months who were hospital patients with acute and chronic infections were studied similarly. While fed breast milk, their average gastric pH was 4.74. After a feeding of undiluted whole milk the pH was 5.35, slightly higher than in the normal infants discussed above. These authors reported that at pH 5 marked inhibition of the growth of members of the colon, dysentery and typhoid group of bacilli occurs. At pH 4 complete inhibition or death of these microorganisms takes place.

Bartholomew et al. (1980) studied microbial growth and nitrite formation in the stomachs of an unspecified number of patients with pernicious anemia and 31 ulcer patients being treated with a drug to reduce gastric acidity. Details of their assays were not given. They found a relation between the pH of the gastric contents and the numbers of bacteria present. Also a close relationship was found between the pH and the percentage of nitrate reduced to nitrite in the gastric contents. They concluded that when the pH is above 5 there is a relatively rich flora and a relatively high nitrate reductase activity.

3. Oxidation of Hemoglobin by Nitrite

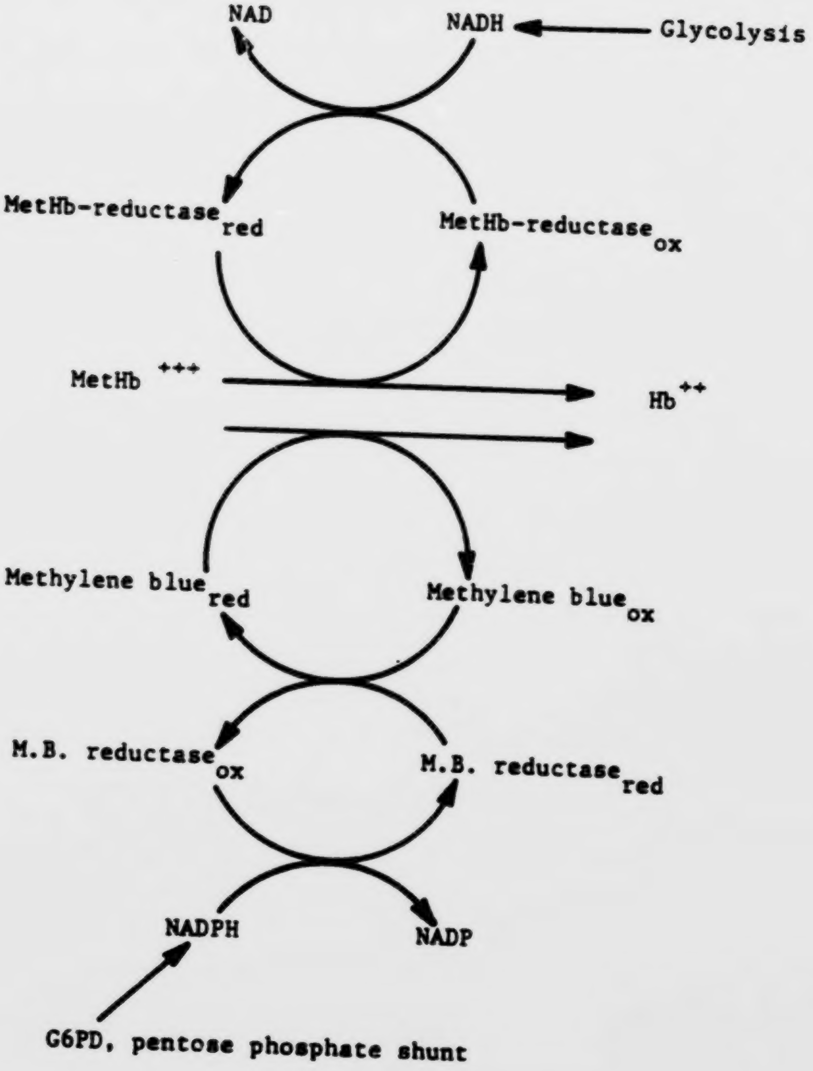
The main fate of nitrite in blood is reaction with hemoglobin to yield methemoglobin. Burrows (1979) characterized the action of nitrite in blood on hemoglobin using mature ewes of mixed breeding. A 3% solution of sodium nitrite in sterile physiological saline was administered intravenously in doses of 6.6-100 mg/kg (1.3-20.3 mg/kg as nitrogen) to groups of 4 ewes. The nitrite ion oxidized divalent ferrous hemoglobin to trivalent ferric methemoglobin, which is incapable of transporting molecular oxygen. The administration of various doses of sodium nitrite resulted in the gradual formation of up to 80% methemoglobin. Doses of 6.6, 22, 35, or 50 mg/kg resulted in maximum methemoglobin levels of 13% at 15 minutes, 43% at 45 minutes, 63% at 60 minutes, or 80% just before death after 60 minutes, respectively.

The normal methemoglobin level in human blood is variously reported between 0.5 and 2.5% of the total hemoglobin. However, Skrivan (1971) determined methemoglobin levels of 100 pregnant women at 2-week intervals. There was a consistent elevation of methemoglobin concentration from the 14th week of pregnancy through delivery, with the average level rising from 2% at the 12th week to 10.5% at the 30th week. This was followed by a decline to 5.5% at the 38th week. All levels returned to normal after delivery. Women around the 30th week of pregnancy would be unusually sensitive to nitrite induction of clinical methemoglobinemia.

4. Reduction of Methemoglobin in Blood

The major system responsible for methemoglobin reduction in mammalian red cells is methemoglobin reductase or diaphorase. As indicated in Figure III-1, this intracellular enzyme requires NADH as a cofactor. Normal human and most mammalian erythrocytes possess a second reductive system that requires NADPH as a cofactor. In most species, the system appears to be dormant, and it is activated only in the presence of exogenously added electron carriers such as methylene blue. The enzyme reduces methylene blue which in turn nonenzymatically reduces methemoglobin (Burrows 1979).

There are large species differences in the rate of reaction of nitrite with hemoglobin to form methemoglobin. Formation is paralleled by similar differences in the rate of reduction by diaphorase to form functional hemoglobin (Smith and Beutler 1966). This is important in determining how sensitive a species is to the vasodilator effects of nitrite and resulting cardiovascular collapse. Species which rapidly remove nitrite from the plasma by reaction with hemoglobin (e.g., ruminants) do not show cardiovascular effects. Species which remove nitrite by this mechanism very slowly (e.g., horses) die of cardiovascular collapse before forming enough methemoglobin to appear cyanotic. Humans fall between these species in removal rate and exhibit both kinds of symptoms concurrently. Different individuals respond with varying sensitivity.


NAD
NADH
Glycolysis
MetHb-reductase$_{red}$
MetHb-reductase$_{ox}$
MetHb $^{+++}$
Hb^{++}
Methylene blue$_{red}$
Methylene blue$_{ox}$
M.B. reductase$_{ox}$
M.B. reductase$_{red}$
NADPH
NADP
G6PD, pentose phosphate shunt

The spontaneous (NADH) and the dormant (NADPH) methemoglobin reductase systems. Methemoglobin (MetHb) reductase is active in intact red cells in the presence of substrates that can generate NADH. The NADPH system requires intact red cells, glucose or its metabolic equivalent, a functioning pentose phosphate shunt, and methylene blue (M.B.). M.B. reductase reduces M.B., which in turn nonenzymatically reduces MetHb. Adapted from Smith (1980).

Figure III-I Reduction of Methemoglobin

Gruener et al. (1973) determined activity of methemoglobin reductase in six pregnant rats (strain not given), 10 rat fetuses, 49 pregnant women, and cord blood of 69 human newborns. The activity of rat fetus methemoglobin reductase was nearly ten times higher than that of adult rats or that found in human cord blood. Human adult blood had 1.5 times the activity of the human cord blood. These findings indicate the possibility that the human fetus might have a weaker defense mechanism against intrauterine exposure to nitrites than that detected in rats. In rats, nitrite has been shown to cross the placenta (Shuval and Gruener 1972).

5. Reactions of Nitrite in the Stomach

Sander et al. (1968) demonstrated the reaction of nitrite with secondary amines to form nitrosamines in the stomachs of Sprague-Dawley rats. Twenty-four rats were fed a standard diet (amount not reported) with 0.01% diphenylamine and 0.15% sodium nitrite and killed after 30, 60, 120, or 180 minutes. Stomach contents were extracted with dichloromethane for two-dimensional chromatography to detect diphenylnitrosamine. The amount found reached a maximum of 31% of theoretical at 120 minutes, presented as 37 µg/g of stomach contents. In vitro studies indicated that the reaction is optimum at pH 1 to 3. This corresponds to the conditions found in the stomach of humans and various experimental animals. Weakly basic amines were converted into nitroso compounds up to a thousand times more rapidly than strongly basic amines, which could not be shown to nitrosate in the rat stomach.

Mirvish et al. (1973) determined the kinetics of nitrosation of the amino acids proline, hydroxyproline and sarcosine by sodium nitrite *in vitro* using perchloric acid to control the reaction pH. The authors found a pH optimum of 2.5. Thiocyanate increased the rate of sarcosine nitrosation 10- to 400-fold, especially at low pH.

Ohshima and Bartsch (1981) determined endogenous formation of N-nitrosoproline in a male volunteer who ingested 0-325 mg nitrate (0-73.5 mg as nitrogen) in red beet juice (0-4.64 mg/kg) followed 30 minutes later by 0-500 mg proline (0-7.14 mg/kg). Urine was monitored for N-nitroso compounds. The amount of nitrosoproline excreted was found to be proportional to the proline dose and increased exponentially with the nitrate dose ingested. Neither nitrate nor proline taken alone led to a detectable increase. The man's normal excretion of nitrosoproline was <3 μg/L. Administration of nitrate followed by proline increased this to a maximum of 17-30 μg/L at the highest doses tested. This was a good test system for demonstrating nitrosation in the human stomach because proline is an amino acid found in most protein foods, and nitrosoproline is neither volatile nor carcinogenic in animals.

Fine et al. (1982) compared the *in vivo* data of Ohshima and Bartsch (1981) with the *in vitro* data of Mirvish (1975). The *in vivo* data fall within theoretical limits predicted if 0.5% to 1.5% of ingested nitrate is reduced to nitrite per hour in human saliva. This study indicates that the extent of *in vivo* formation of other more harmful N-nitroso compounds in the human stomach can be predicted from *in vitro* kinetic studies.

Archer et al. (1975) demonstrated the ability of ascorbic acid to block nitrosation of morpholine in vitro by rapidly reducing nitrite to nitric oxide or nitrogen. The reaction kinetics are dependent on the redox potential of the medium. Under anaerobic conditions, one mole of ascorbate will reduce two moles of nitrite. In the presence of bubbling air equimolar ascorbate is required. These experiments were conducted at pH 3, 3.5 and 4 maintained by perchloric acid.

Similar in vitro studies were done by Kamm et al. (1977) showing the ability of α-tocopherol (vitamin E) to remove nitrite ion from simulated gastric fluid. The rate of reaction was shown to be optimum at pH 2 to 3 and negligible at pH 5. The α-tocopherol reacted more readily than ascorbate to destroy nitrite ion at pH values below 4. Appropriate controls were used by these authors.

Ohshima and Bartsch (1981) confirmed the inhibitory value of ascorbic acid and α-tocopherol in the human volunteer protocol presented above. Intake of 1 gram of ascorbic acid simultaneously with 325 mg nitrate (73.5 mg as nitrogen) and 250 mg proline reduced the nitrosoproline concentration in urine to the control level, whereas 500 mg of α-tocopherol inhibited nitrosation about 50%.

D. Excretion

Keith et al. (1930) studied excretion of nitrates in four young men. After ingestion of 10 grams of ammonium nitrate, a measurable amount of nitrate could not be detected in the feces. Seventy-nine percent of the nitrate ingested was excreted promptly, and a total of 88% was recovered within 3-4 days. Nitrate was assayed with a colorimetric test using diphenylbenzidine.

Tannenbaum et al. (1980) recorded urinary excretion of nitrate by individuals on a low-nitrate soy diet for 84 days. The average daily excretion was about six times the nitrate intake. The data suggest a fairly constant introduction of extra-dietary nitrate into the system. Based on these data and the authors' previous demonstration of nitrite in ileostomy contents in the absence of nitrate, the authors conclude that the most likely explanation is that gut microorganisms oxidize reduced forms of nitrogen, such as ammonium compounds, to nitrite in the intestinal tract. This nitrite is absorbed and is then converted to nitrate by the process of methemoglobin formation and subsequent reduction.

Green et al. (1981) studied nitrate excretion in germ-free or conventional male Sprague-Dawley rats weighing 150 grams. The basal diet was supplemented with 0%, 0.001% or 0.005% $Na^{15}NO_3$ (0, 0.5 or 2.5 mg/kg/day; equivalent to 0, 0.08 or 0.4 mg/kg/day as nitrogen). Urine and feces were collected during 24-hour intervals. Nitrate content was determined by reducing it to nitrite and assaying by diazotization and coupling with a Griess reagent. Isotope levels were determined by conversion to nitrobenzene and analysis by gas chromatography-mass spectrometry. Rats not supplemented with sodium nitrate excreted ten times more nitrate in the urine than they ingested. There was no difference between germ-free and conventional rats. This indicates that endogenous nitrate synthesis is not due to intestinal flora. Rats supplemented with nitrate at the lower dose (0.5 mg/kg/day) excreted slightly more nitrate than they ingested. The rats supplemented at the higher dose (2.5 mg/kg/day) excreted slightly less nitrate than they ingested. The possibility of a body pool of nitrate turning over during the experiment was ruled out. After stopping supplementation no $^{15}NO_3$ was detectable in the urine on the third day. The authors could not detect nitrate in feces of conventional rats.

Schneider and Yeary (1975) studied the kinetics of the elimination of nitrite from the plasma of seven dogs of both sexes and mixed breeds, seven cross-bred ewes, and seven Shetland ponies of both sexes. Sodium nitrite was administered intravenously at 20 mg/kg (4.1 mg/kg as nitrogen) and blood was collected 15-30 seconds after injection, at 1-minute intervals between 1 and 10 minutes, at 5 minute intervals between 10 and 30 minutes, at 10 minute intervals between 30 and 60 minutes, and then at 1.5, 2, 3, 6 and 24 hours after injection. A colorimetric assay for nitrite was used after plasma was isolated. The half-life for elimination of nitrite from plasma was between 0.5 and 0.6 hours in all three species. The authors concluded that this rapid clearance indicated a process other than renal excretion, such as metabolic conversion to nitrate.

Davison et al. (1964) added nitrate to the diets of Holstein heifers during pregnancy. Milk samples were taken at weekly intervals from parturition to 30 days. Milk from heifers fed normal rations averaged 5 ppm nitrate, while milk from heifers fed 440 mg/kg/day and 660 mg/kg/day nitrate (equivalent to 99.4 and 149.2 mg/kg/day as nitrogen) averaged 9 ppm and 15 ppm nitrate, respectively. In contrast, Crowley et al. (1974) reported a long-term study of a Wisconsin dairy herd drinking water with 374 ppm nitrate.

The Holstein cows produced milk containing an average of 5.7 ppm nitrate. This compared to controls drinking water containing 19 ppm nitrate which produced milk containing 5.6 ppm nitrate.

E. Bioaccumulation and Retention

No evidence was found for bioaccumulation of nitrate or nitrite in any tissue storage depot. However, nitrate is retained by recycling through saliva with reabsorption into plasma as explained in III.B. (Spiegelhalder et al. 1976).

F. Summary

The primary route of nitrate and nitrite absorption is through the gastrointestinal tract. Nitrate is absorbed by active transport through the wall of the upper small intestine. Nitrite is absorbed by diffusion across the gastric mucosa and also through the wall of the intestinal tract. In both cases, absorption following ingestion is essentially complete (i.e., 100%). Nitrate and nitrite can also be absorbed into the blood through the lungs.

Nitrate is rapidly disseminated throughout the body in the blood and is actively secreted in saliva, gastric juices, and urine. Nitrite reacts so rapidly with hemoglobin that little is transported to the tissues. Nitrate and nitrite do not bioaccumulate. However, they are constantly present in the body as a result of *de novo* synthesis as well as ingestion.

Nitrate is reduced to nitrite by the action of oral and gastrointestinal bacteria. Approximately 5% of ingested nitrate is transformed to nitrite by oral bacteria in healthy human adults and older children. Reduction of nitrate to nitrite also occurs in the stomach of young infants and others with abnormally high pH in the stomach. However, no quantitative data are available on how much ingested nitrate is transformed to nitrite in the stomach. Nitrite that is absorbed into the blood reacts with hemoglobin, oxidizing it to methemoglobin. Nitrite in the stomach may react with secondary amines and other amino substrates present in food or in the epithelium to form N-nitroso compounds.

Nitrate is excreted readily through the kidneys. When excess nitrate is not added to the diet, rats excrete significantly more nitrate than they ingest. Nitrite is so reactive that none remains to be excreted.

IV. HUMAN EXPOSURE

Nitrate is a normal component of the human diet. A typical daily intake by an adult in the United States is about 75 mg/day (about 0.2-0.3 mg nitrate-nitrogen/kg/day) (NAS 1981). Of this, over 85% comes from the natural nitrate content of vegetables such as beets, celery, lettuce and spinach. Daily intakes of nitrate by vegetarians may exceed 250 mg/day (0.8 mg nitrate-nitrogen/kg/day) (NAS 1981). The contribution from drinking water is usually quite small (about 2%-3% of the total) (NAS 1981), but may increase to about 50% if water containing 10 mg nitrate-nitrogen is consumed (USEPA 1990). The USEPA has performed a thorough investigation of human exposure to nitrate/nitrite through drinking water. The findings are presented in a document entitled, "Estimated National Occurrence and Exposure to Nitrate/Nitrite in Public Drinking Water Supplies" (USEPA 1990).

V. HEALTH EFFECTS IN ANIMALS

The adverse health effects of nitrate are largely due to its conversion to nitrite. This conversion is mediated by bacteria which are present in the alimentary tract. Thus, equal doses of nitrate often do not produce equal effects in different species, due to differences in the number and type of enteric bacteria. For example, ruminants are especially sensitive to nitrate due to the high bacterial content of the rumen (Emerick 1974), while dogs appear to reduce nitrate only slowly (Greene and Hiatt 1954). For these reasons, studies in ruminants or dogs are not considered here, since dose-response data are not quantitatively applicable to humans. Similarly, dose-response data from other animal species (rats, mice, etc.) should be extrapolated to humans with caution, due to possible differences in nitrate reduction between species.

A. Short-Term

1. Lethality

Lethal dose data reported for nitrate and nitrite in various species are summarized in Table V-1. As may be seen, nitrate has low acute lethality (only slightly more than potassium or sodium chloride), and probably causes death mainly by electrolyte imbalances (ECETOC 1988). In contrast, nitrite causes lethality at about 1/10 the dose of nitrate. This is presumably due to either massive methemoglobinemia and/or severe hypotension (see below). It is

Table V-1 Oral LD_{50} of Nitrate and Nitrite

Animal	Cation	LD_{50} for NO_3^-		LD_{50} for NO_2^-		Reference
		mg NO_3/kg	mg N/kg	mg NO_2/kg	mg N/kg	
Rat	Na+			100	30	a
Rat	Na+			120	36	b
Rat	K+	1,986	457			c
Rat	K+			46	14	c
Rat	Na+			105	30	d
Rat (1-yr)	Na+			51-87	15-26	e
Rat (3-mo)	Na+			73	22	e
Mouse	K+			119	36	c
Mouse	K+			95	28	c
Rabbit	K+	1,166	268	108	32	b
Rabbit	Na+	1,955	450			b

[a]Imaizumi et al. 1980
[b]Merck Index 1976
[c]WHO 1962
[d]Imaizumi et al. 1980
[e]Druckrey et al. 1963

important to note that these LD_{50} values refer to large single doses of compound, and that administration of equal or even greater doses spread out over the course of a day in food or water do not result in lethality (Shuval and Gruener 1977, Imaizumi et al. 1980).

2. Methemoglobinemia

Nitrate

No short-term studies were located on the effect of nitrate ingestion on methemoglobinemia levels in animals.

Nitrite

Shuval and Gruener (1977) administered single oral doses of 0, 2.5, 5, 10, 15, 20, 25 or 30 mg/kg of $NaNO_2$ to pregnant rats. This corresponds to doses of 0, 0.5, 1, 2, 3, 4, 5 or 6 mg nitrite-nitrogen/kg/day. There was a clear dose-response increase in methemoglobin levels in the dam, ranging from 0.9% in controls to 60% in animals given 30 mg/kg $NaNO_2$. Methemoglobin levels in the fetuses also increased, but were about 50% those of the dam.

Shuval and Gruener (1977) supplied groups of fifteen 50-day-old male C_{57}bl/6J mice with drinking water containing 0, 100, 1,000, 1,500 or 2,000 mg sodium nitrite/L for 3 weeks. These levels produced doses of 0, 2.6, 26, 40 or 53 mg nitrite-nitrogen/kg/day. No change in methemoglobin level was observed at 2.6 mg nitrite-nitrogen/kg/day. An increase was observed in animals receiving 26 mg nitrite-nitrogen/kg/day, but this was not statistically significant. Doses of 40 and 53 mg nitrite-nitrogen/kg/day both produced a significant increase in methemoglobin levels ($P<0.05$).

Shuval and Gruener (1972, 1977) supplied groups of 12 pregnant rats (strain "sabra") with water containing 0, 2,000 or 3,000 mg/L sodium nitrite through the 3rd week after parturition. This corresponded to doses of 1, 54 or 81 mg nitrite-nitrogen/kg/day. Mean methemoglobin values were 1.1%, 5.5% and 24% for the females in the control, low-dose, and high-dose groups, respectively. From birth to weaning, pups in the treated groups showed no abnormally high methemoglobin, but total hemoglobin was 20% lower than controls.

Imaizumi et al. (1980) studied the effect of sodium nitrite on methemoglobin formation in rats. Sprague-Dawley rats (200 g) were given single oral doses of $NaNO_2$ by gastric intubation at dose levels of 0, 10, 25, 50, 100 or 150 mg/kg (equivalent to 0, 2, 5, 10, 20 or 30 mg nitrite-nitrogen/kg). Within 1 hour after administration of the high dose, methemoglobin concentrations increased to 45%-80%. Methemoglobin levels subsequently returned to control levels (approximately 2%) at 24 hours. A clear dose-response relationship was apparent between nitrite dose and methemoglobin levels, with maximum levels of 10%, 40%, 70%, and 80% at doses of 25, 50, 100 and 150 mg/kg, respectively.

3. Effects of Blood Pressure

Nitrite ion is a smooth muscle relaxant that can cause vasodilation and hypotension. No studies were located on vasodilation following oral exposure to nitrite ion, but several studies investigated hypotensive effects following intravenous injection of $NaNO_2$. For example, Constantine et al. (1971) injected 12 dogs with 4 mg/kg of $NaNO_2$ (0.8 mg nitrite-nitrogen/kg), and observed an average decrease in blood pressure of 26 mmHg. Similarly, Klimmek et al. (1983) injected 5 dogs with 15 mg/kg of $NaNO_2$ (3 mg nitrite-nitrogen/kg/day), and observed an average increase of 50% in blood flow to the brain (indicative of cerebral vasodilation). Similar effects are produced by organic nitrates (e.g., amyl nitrate, nitroglycerin), but not by inorganic nitrate ion (Nickerson 1975).

4. Histopathological Effects

Nitrate

No studies were located regarding histopathological effects resulting from short-term ingestion of nitrate by animals.

Nitrite

Inai et al. (1979) exposed groups of 10 male and 10 female mice to $NaNO_2$ in drinking water for 6 weeks. Concentration levels were 0, 500, 1,250, 5,000 or 10,000 mg NO_3/L (corresponding to doses 0, 15, 37, 150 or 300 mg nitrite-nitrogen/kg/day). Microscopic examination of visceral organs revealed slight degeneration and spotty necrosis of hepatocytes in liver, along with hemosiderin deposition in liver, spleen and lymph nodes. This was judged to be secondary to a hemolytic effect of $NaNO_2$. No data were provided regarding dose-response relationships for these effects.

Nitrite plus Nitrosatable Substrates

Asahina et al. (1971) studied the acute toxicity of sodium nitrite and dimethylamine in adult male Charles River mice. Liver necrosis was observed when 1,000 mg/kg dimethylamine and 100 mg/kg sodium nitrite (20.3 mg nitrite-nitrogen/kg) were given simultaneously by gavage. The necrosis was attributed to intragastric formation of nitrosodimethylamine. Liver injury was reduced when the nitrite was given 15-120 minutes before, but not 3 hours after, the dimethylamine. This suggests that nitrite disappeared fairly rapidly from the stomach.

Lijinsky and Greenblatt (1972) exposed rats by gavage to 1.0 mL doses of aminopyrine (35 mg/mL), $NaNO_2$ (5-40 mg/mL) or both aminopyrine (35 mg/mL) and nitrite (5-40 mg/mL). These doses of $NaNO_2$ correspond to 3-24 mg nitrite-nitrogen/kg/day. After 48 hours, animals were killed and the livers examined histologically. There was a clear dose-dependent increase in the occurrence of hepatic necrosis as a function of nitrite level in the presence of aminopyrine, while no effect was observed following exposure to aminopyrine (35 mg/mL) or $NaNO_2$ (40 mg/mL) alone. The reaction of nitrite plus aminopyrene is known to yield dimethylnitrosamine _in vitro_ (Lijinsky 1974), and Lijinsky and Greenblatt concluded that the hepatic effects were the result of dimethylnitrosamine formation _in vivo_.

Acute nitrosamine toxicity in the liver has been prevented by simultaneous administration of ascorbic acid with the nitrite and amine (Greenblatt 1973, Kamm et al. 1975). Ascorbate prevents toxicity only when it is present in the stomach of the rat with the amine and nitrite. Erythorbic acid (Kamm et al. 1975) and an emulsion of α-tocopherol (vitamin E) (Kamm et al. 1977) are as effective as ascorbic acid in preventing hepatic necrosis. Complete protection from measurable liver toxicity (elevation of SGPT) required that the inhibitor be at least equimolar with the nitrite (Kamm et al. 1977). Prevention of histologically observed necrosis required twice the amount of ascorbic acid (Greenblatt 1973). The vitamins are oxidized in the process of inactivating nitrite. The common antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) had no measurable protective value at 0.6 equimolar (Astill and Mulligan 1977).

5. Effects on Thyroid Function

Wyngaarden et al. (1953) studied the effects of high levels of nitrate in drinking water on the iodide-concentrating mechanism of the thyroid gland. Nitrate appeared to compete with iodide in uptake by the thyroid, leading to a decreased iodide uptake. Horing et al. (1985) and Seffner et al. (1985) (as reported in ECETOC 1988) exposed rats to drinking water containing 40, 200, 1,200 or 4,000 mg/L of nitrate for 100 days. This corresponds to doses of 0.9, 4.6, 28 or 92 mg nitrate-nitrogen/kg/day. Thyroid function was examined by ^{131}I-uptake, ^{131}I-serum level, ^{131}I-corporation into proteins, the thyroid gland weight and histopathological parameters. Thyroid weight and ^{131}I-uptake appeared to be changed slightly at all dosage levels. Histopathological parameters were altered at all dose levels but there was no dose-response relationship in the finding.

Jahreis et al. (1987) investigated the effect of nitrate on thyroid hormone production in young pigs. Groups of 7 animals were exposed to 0 or 3% KNO_3 in the diet for 2 days. Assuming consumption of 0.04 kg food/kg body weight per day, this corresponds to a dose of 170 mg nitrate-nitrogen/kg/day. Animals exposed to KNO_3 had significantly reduced serum levels of T_4 ($P<0.01$), but not of T_3. Supplementation of the diet with excess iodide (0.5 mg/kg) resulted in increased levels of T_4 in both groups, and the difference between them was not significant. Similar results were described when exposure was continued for 6 weeks, although excess iodide was not able to fully reverse the effect of nitrate. The authors interpreted these observations to show that nitrate inhibited synthesis of thyroid hormones. However, since T_3 is the biologically active form of thyroid hormone, and since T_3 levels were not affected by nitrate exposure, the relevance of these observations is not clear.

B. Longer-Term

1. Methemoglobinemia/Hematology

Nitrate

Shuval and Gruener (1977) supplied groups of 52 male rats with water containing 0 or 2,000 mg/L of $NaNO_3$ (0 or 330 mg/L nitrate-nitrogen) for

16 months. This corresponded to average daily doses of 150-300 mg NO_3/kg/day (34-69 mg nitrate-nitrogen/kg/day). Mean methemoglobin levels were not significantly elevated at any time between 1 and 16 months. This study identifies a NOAEL of 69 mg nitrate-nitrogen/kg/day.

Chow et al. (1980) provided young male rats (8/group) with drinking water containing 0 or 400 mg sodium nitrate/L (equivalent to 0 or 6.6 mg nitrate-nitrogen/kg/day). Rats received this treatment for 16 weeks during which time blood samples were collected for the evaluation of methemoglobin. Methemoglobin concentrations fluctuated between 0 and 1.2%. Although no statistical evaluation was reported, this was the same range of concentrations reported for controls. This study identifies a NOAEL of 6.6 mg nitrate-nitrogen/kg/day.

Chow et al. (1980) studied the effects of chronic nitrate exposure on methemoglobin concentration in male Sprague-Dawley rats. Rats were given 0 or 4,000 mg sodium nitrate/L (an amount equivalent to 0 or 66 mg nitrate-nitrogen/kg/day) for 14 months. Methemoglobin concentration, measured every other week throughout the course of the study, fluctuated between 0 and 2%. This was not significantly elevated over control values. This study identifies a NOAEL of 66 mg nitrate-nitrogen/kg/day.

Nitrite

Druckrey et al. (1963) exposed 30 male and 30 female rats to $NaNO_2$ in drinking water at a level that resulted in ingestion of 100 mg NO_2/kg/day

(20 mg nitrite-nitrogen/kg/day). Exposure was for 70 days. Methemoglobin levels averaged 7% in the exposed animals compared to 0.8% in controls.

Shuval and Gruener (1972) exposed groups of 8 male rats for 24 months to water containing 0, 100, 1,000, 2,000 or 3,000 ppm of sodium nitrite. This corresponds to doses of about 0, 2, 20, 40 or 60 mg nitrite-nitrogen/kg/day. There were no significant differences in growth or mortality between the groups. Methemoglobin levels were increased to 5%, 12% and 22% in the 1,000, 2,000 and 3,000 ppm dose groups, respectively.

Chow et al. (1980) provided young male rats (8/group) with drinking water containing 0 or 200 mg sodium nitrite/L (equivalent to 0 or 4 mg nitrite-nitrogen/kg/day). Rats received this treatment for 16 weeks during which time blood samples were collected for the evaluation of methemoglobin. Methemoglobin concentration fluctuated between 0.5% and 3.1% compared to control methemoglobin values of less than 1.2%. Statistical evaluation of these data was not reported.

Chow et al. (1980) measured the effects of chronic nitrite exposure on methemoglobin concentration in male Sprague-Dawley rats. Rats were provided with drinking water containing 0 or 2,000 mg sodium nitrite/L (equivalent to 0 or 40 mg nitrite-nitrogen/kg/day) for 14 months. Blood was analyzed for methemoglobin every other week throughout the course of the study. Methemoglobin in nitrite-treated rats fluctuated from 1% to 35%, and was significantly greater than the methemoglobin concentration (1%) in animals receiving water. This study identifies a LOAEL of 40 mg nitrite-nitrogen/kg/day but does not identify a NOAEL.

Imaizumi et al. (1980) studied the effects of long-term ingestion of sodium nitrite on methemoglobin concentration and other hematological parameters in the rat. Four Sprague-Dawley rats (200 g) were given drinking water containing 0 or 5,000 mg/L $NaNO_2$ for 6 months. The average daily intake of $NaNO_2$ was 50 mg nitrite-nitrogen/kg/day. Fluctuations in methemoglobin occurred as a function at the time of sampling, a phenomena attributed to the nocturnal water consumption patterns of the rat. Maximum methemoglobin concentrations (ranging from 33% to 88%) were observed between 6:00 and 9:00 p.m., and minimum concentrations (ranging from 4% to 19%) at 9:00 a.m. The 6 month average methemoglobin level measured at 3:00 p.m. was 8.2% compared to 1.0% in control rats. No abnormalities in growth or development were observed in the treated group compared to controls. Although hematological effects other than methemoglobinemia (erythrocytic Heinz bodies, anisocytosis and hypohemoglobinemia) were seen in the treated rats, the extent of these effects were not described.

Til et al. (1988) investigated the subchronic oral toxicity of potassium nitrite in rats. Groups of 10 males and 10 females were given drinking water containing 0, 100, 300, 1,000 or 3,000 mg/L of KNO_2 for 13 weeks. This corresponds to doses of 0, 1.7, 5, 17 or 50 mg nitrite-nitrogen/kg/day. All solutions were adjusted (by addition of KCl) so that each contained the same final concentration of potassium (35 mM). Methemoglobin levels increased from 1.7%-2.3% in the control group to 5.7%-7.6% in the high-dose group ($P<0.05$). No significant increases in methemoglobin were noted at lower doses. This study identifies a NOAEL of 17 and a LOAEL (based on methemoglobin) of 50 mg nitrite-nitrogen/kg/day.

Grant and Butler (1989) administered $NaNO_2$ to groups of 50 male F344 rats for 115 weeks. Dose levels were 0, 0.2 or 0.5% in the diet, corresponding to intakes of about 20 or 50 mg nitrite-nitrogen/kg/day. Transient effects were observed at both dose levels for a number of hematological parameters, including decreased red blood cell counts, mean red cell volume, hematocrit and hemoglobin concentration. These effects developed slowly over the first 8 weeks of the study, then tended to return to near normal by 52 weeks. Methemoglobin levels were not measured. Based on the changes in blood, this study identifies a chronic oral LOAEL of 20 mg nitrite-nitrogen/kg/day. A NOAEL was not identified.

2. Histopathological Effects

Nitrate

Sugiyama et al. (1979) fed groups of 50 male and 50 female mice sodium nitrate at dose levels of 0, 2,500 or 5,000 mg/kg/day (0, 410 or 820 mg nitrite-nitrogen/kg/day) for more than a year. Amyloidosis was present in 37% of survivors at the high dose, in 42% at the lower dose, and in 25% of the controls. The authors reported that the liver was more affected by sodium nitrate than other organs and showed marked atrophy and hemosiderosis. Amyloidosis, however, was noted in various organs including the liver, spleen, kidneys and adrenals. Quantitative data on these lesions were not reported.

Chow et al. (1980) provided young male rats (8/group) with drinking water containing 0 or 400 mg sodium nitrate/L (equivalent to 0 or 6.6 mg nitrate-nitrogen/kg/day). No animal mortality occurred during the course of treatment. At the completion of 16 weeks of treatment, rats were sacrificed and histologically examined. Liver, kidney, spleen, heart and body weights were not altered in treated animals. Lung weights in the nitrate-treated rats averaged 1.7 g compared to 1.5 g in control rats. No other statistical or histopathologic details were reported.

Chow et al. (1980) measured the effects of chronic nitrate exposure on histopathology in male Sprague-Dawley rats. Rats were provided drinking water containing 0 or 4,000 mg sodium nitrate/L (equivalent to 0 or 66 mg nitrate-nitrogen/kg/day) for 14 months. Four of the 10 rats given nitrate for 14 months survived. Nitrate-treated rats had lighter livers and heavier lungs than the control group, and moderate lung lesions were observed in all of the surviving rats. This study identifies a LOAEL of 66 mg nitrate-nitrogen/kg/day.

Fritsch et al. (1980) supplied groups of ten male Sprague-Dawley rats with a diet containing 0, 0.5 or 5% nitrate for 6 months. This corresponds to doses of about 0, 50 or 560 mg nitrate-nitrogen/kg/day. Changes were observed in the spleen in all the treated animals. The changes included a significant release of ferric iron and the presence of hemorrhagic areas. Further details on the magnitude or severity of these changes were not provided. These changes may be secondary to the hematological effects of nitrite.

Nitrite

Druckrey et al. (1963) exposed 30 male and female rats for their lifetime to $NaNO_2$ in drinking water at a dose rate of 100 mg/kg/day (20 mg nitrite-nitrogen/kg/day). The lifespan of the treated animals was decreased to an average of 630 days as compared to 730 days for the control animals. Body weights for the treated rats were 11% below those of the controls. This was attributed to a decreased willingness of the exposed rats to drink the water. No treatment-related histopathological effects were noted in liver, kidney, lungs or spleen.

Shuval and Gruener (1972) exposed groups of 8 male rats for 24 months to water containing 0, 100, 1,000, 2,000 or 3,000 ppm of sodium nitrite. This corresponds to doses of about 0, 2, 20, 40 or 60 mg nitrite-nitrogen/kg/day. There were no significant differences in growth or mortality between the groups. Histological examination of the lungs revealed dilated bronchi and the mucosa and muscles were often atrophied. Interstitial fibrosis and emphysema were also observed. These changes were found with increasing frequency and severity in the 1,000, 2,000 and 3,000 ppm groups. Histological examination of the heart revealed an increased percentage of coronary arteries that were characterized as "thin and dilated." Similar effects on the coronary arteries were reported in a follow-up study by Shuval and Gruener (1977) at exposure levels of 200, 1,000, 2,000 or 3,000 mg/L of $NaNO_2$. Exposure to 2,000 mg/L of $NaNO_3$ also produced the same effect. However, it is not clear how many samples or how many vessels were examined from each animal, so the LOAEL for this effect cannot be defined from the data presented. Also, this effect appears to be at least partly due to the absence of coronary artery thickening and narrowing that normally occurs in aged rats, so it is not certain that these changes are inherently adverse. These studies identify a NOAEL of 2 and a LOAEL (based on effects on the lung) of 20 mg nitrite-nitrogen/kg/day.

Inai et al. (1979) exposed groups of 50 male and 50 female mice to drinking water containing 0, 1,250, 2,500 or 5,000 mg sodium nitrite/L (0, 41.6, 83.2 or 166.6 mg nitrite-nitrogen/kg/day) for more than 18 months. Histological examinations revealed marked atrophy and hemosiderosis in liver, and amyloidosis in liver, spleen, kidneys and adrenal. However, no data were presented on the doses which caused these effects.

Chow et al. (1980) provided young male rats (8/group) with drinking water containing 0 or 200 mg sodium nitrite/L (equivalent to 0 or 4 mg nitrite-nitrogen/kg/day). At the completion of 16 weeks of treatment, rats were sacrificed and histologically examined. No animals deaths occurred as a result of the treatment. The lungs of rats given nitrite averaged 2.0 g compared to the average lung weight of 1.5 g of control rats. The authors did not report whether this difference was statistically significant. Liver, heart, kidney, spleen and body weight were not affected by treatment with nitrite. No other histopathological details were reported.

Chow et al. (1980) measured the effects of chronic nitrite exposure on histopathology in male Sprague-Dawley rats. Rats were provided drinking water containing 0 or 2,000 mg sodium nitrite/L (equivalent to 0 or 40 mg nitrite-nitrogen/kg/day) for 14 months. Surviving animals (5/12) were found to have significantly increased lung weights when compared to controls or animals given nitrate. Examination of lung tissue revealed chronic pneumonitis (microabscesses, congestion) in all nitrite-exposed rats. This study identifies a LOAEL of 40 mg nitrite-nitrogen/kg/day but does not identify a NOAEL.

Fritsch et al. (1980) supplied groups of ten male Sprague-Dawley rats with a diet containing 0, 0.05 or 0.5% $NaNO_2$ for 6 months. This corresponded to doses of 0, 6.3 or 74 mg nitrite-nitrogen/kg/day. Changes were observed in the spleen in all the treated animals. The changes included a significant release of ferric iron and the presence of hemorrhagic areas. Further details were not provided on the magnitude or the severity of these changes.

Til et al. (1988) investigated the subchronic oral toxicity of potassium nitrite in rats. Groups of 10 males and 10 females were given drinking water containing 0, 100, 300, 1,000 or 3,000 mg/L of KNO_2 for 13 weeks. This corresponds to doses of 0, 1.7, 5.0, 17 or 50 mg nitrite-nitrogen/kg/day. All solutions were adjusted (by addition of KCl) so that each contained the same final concentration of potassium (35 mM). Water intake tended to decrease in a dose-dependent fashion, although this was statistically significant only in the two highest doses. Complete histological examination revealed no pathological changes in any tissue at any dose, except for a very-slight to slight hypertrophy of the zona glomeruloa in animals supplied 300 mg/L or higher. It seems likely that this response is a normal endocrine adaptation to a change in blood volume and/or blood pressure resulting from the decreased water intake, and is not judged to constitute an adverse health effect.

3. Other Effects

Ten male Sprague-Dawley rats initially weighing 50 grams were fed 2,500 mg/kg/day sodium nitrate (412.5 mg/kg/day nitrate-nitrogen) in the diet for 6 months. The results showed a statistically significant ($P<0.01$)

diuretic effect by 2 months when compared to rats fed equimolar sodium chloride. Rats fed one-tenth of that dose showed no significant diuresis (Fritsch et al. 1980).

C. Reproductive/Teratogenicity Effects

Nitrate

The U.S. Food and Drug Administration sponsored extensive tests of the reproductive and teratogenic effects of $NaNO_3$ and KNO_3 in mice, rats, hamsters and rabbits (FDA 1972a, b). Groups of 20-26 mice, rats or hamsters and 10-13 rabbits were treated by gavage on days 6-15 (mice, rats), days 6-10 (hamster) or days 6-18 (rabbits) of gestation. Fetuses were delivered by Cesarean section, and examined for visceral and skeletal malformations. Dose levels (expressed as nitrate-nitrogen) ranged from 0.6 to 66 mg/kg/day for mice, from 0.3 to 41 mg/kg/day for rats, from 0.4 to 66 mg/kg/day for hamsters and from 0.3 to 41 mg/kg/day for rabbits. No significant effects were detected regarding maternal reproductive parameters (percent pregnant, abortion frequency, number of litters), fetotoxicity (percent fetal resorptions, live fetuses per dam, average fetal weight) or fetal malformations up to the maximum doses administered to each species. These studies identify a reproductive/developmental NOAEL of 66 mg nitrate-nitrogen/kg/day for mice and hamsters and 41 mg nitrate-nitrogen/kg/day for rats and rabbits.

Sleight and Atallah (1968) studied the effects of nitrate on reproduction and development in guinea pigs. Groups of 3-6 females were exposed to drinking water containing 0, 300, 2,500, 10,000 or 30,000 ppm KNO_3 for

143-204 days. Based on measured water intake and body weight, this resulted in average doses of 0, 12, 102, 507 or 1,130 mg nitrate-nitrogen/kg/day. Normal conception occurred at all dose levels. Since males were also exposed, their fertility was not impaired. No statistically significant effect on reproductive performance (litters produced, number of live births) was detected except in the high dose group, where there was a decrease in number of live births (only 2 from 3 females, compared with 31 from 4 females in the control group). Statistical significance was not reported. One female in this group died, and had 4 mummified fetuses in utero. The authors attributed the fetal effects to hypoxia due to maternal methemoglobinemia, although data on this were not provided. No fetotoxic malformations were observed at any dose. This study identifies a reproductive NOAEL of 507 and a LOAEL of 1,130 mg nitrate-nitrogen/kg/day.

Alavantic et al. (1988b) exposed male C3H X 101 F_1 mice (5/group) to 0, 600 or 1,200 mg/kg/day sodium nitrate (99 or 198 mg nitrate-nitrogen/kg/day) by stomach intubation for 3 days. Mice were sacrificed 11 and 17 days following treatment and 500 sperm cells per animals from the caudal epididymis were examined for abnormal morphology. No increase in sperm-head abnormalities were observed in mice receiving sodium nitrate at 600 mg/kg/day. A slight increase (not significant) was noted in mice receiving 1,200 mg/kg/day. This study identified a NOAEL for sodium nitrate of 600 mg/kg/day for sperm-head abnormally in mice.

Alavantic et al. (1988a) investigated the effect of sodium nitrate on reproduction in mice. Male and female C3H X 101 mice (10-12 weeks old, 25 per group) were dosed intragastrically with 600 or 1,200 mg/kg/day $NaNO_3$ (99 or

198 mg nitrate-nitrogen/kg/day) daily for 14 days prior to mating. Fertility, litter size and sex ratio were evaluated and the degree of heritable translocations and sperm abnormalities were examined in both parents and offspring. Fertility and litter size were not reduced in the mice receiving either 600 or 1,200 mg/kg/day $NaNO_3$. Cytogenetic evaluation of the spermatocytes of parental mice revealed a significant increase in sex chromosome univalency at both doses. Sperm-head abnormalities were significantly increased in mice receiving 1,200 mg/kg/day but not at the low dose group. No effect was evident at either dose level on testes weight or body weight. Examination of F_1 males revealed no heritable chromosomal translocations, induction of sex chromosomal univalency, or increase in the number of sperm-head abnormalities at either dose. This study identifies a LOAEL of 600 mg/kg/day $NaNO_3$ for chromosomal changes in mouse germ cells.

Markel et al. (1989) investigated the effect of nitrate exposure on the development of sensory-motor function and learning behavior in the rat. Drinking water containing 0, 1.12 or 2.24 mM KNO_3 (120 or 240 mg KNO_3/L) was provided to pregnant Wistar rats through gestation and lactation. This corresponds to doses 0, 1.7 or 3.4 mg nitrate-nitrogen/kg/day. At 21 days postpartum (weaning), the offspring were also provided the nitrate-containing water. Sensory-motor parameters evaluated included righting reflex and cliff-avoidance, open and closed-field locomotor behavior, homing behavior, eye-opening and startle. Behavioral parameters included one-way active avoidance, and water rewarded discrimination learning. Nitrate-exposed rats developed mature righting and cliff-avoidance reflexes and hearing startle reaction earlier than controls. Motor activity (open field) was increased soon after birth in treated animals but at day 20 hypoactivity was reported.

As adults, treated rats showed deficits in rewarded discriminitive learning tests and in establishing active avoidance response (P<0.001). This study identifies a LOAEL of 1.12 mM KNO_3 (17 mg nitrate-nitrogen/L) for neurobehavioral development in the rat, although the biological significance of the observed changes are not certain.

Nitrite

The U.S. Food and Drug Administration sponsored extensive tests of the reproductive and teratogenic effects of $NaNO_2$ and KNO_2 in mice, rats, hamsters and rabbits (FDA 1972c,d). Groups of 20-26 mice, rats or hamsters and 10-13 rabbits were treated by gavage on days 6-15 (mice, rats), days 6-10 (hamster) or days 6-18 (rabbits) of gestation. Fetuses were delivered by Cesarean section, and examined for visceral and skeletal malformations. Dose levels (expressed as mg nitrite-nitrogen) ranged from 0.04 to 4.6 mg/kg/day for mice, from 0.1 to 2 mg/kg/day for rats, from 0.04 to 5.3 mg/kg/day for hamsters and from 0.03 to 4.6 mg/kg/day for rabbits. No significant effects were detected regarding maternal reproductive parameters (percent pregnant, abortion frequency, number of litters), fetotoxicity (percent fetal resorptions, live fetuses per dam, average fetal weight) or fetal malformations up to the maximum doses administered to each species. In rats, the highest dose tested produced slight indications of delayed skeletal maturation, especially with respect to ribs and skull. In hamsters, a delay in skeletal maturation, which was not clearly dose related, was observed. These effects are not considered to be of practical health significance. These studies identify a reproductive/developmental NOAEL of 5.3 mg nitrite-nitrogen/kg/day for mice and hamsters, 4.6 mg nitrite-nitrogen/kg/day for rabbits, and 2.0 mg nitrite-nitrogen/kg/day for rats.

Druckrey et al. (1963) supplied rats with $NaNO_2$ in drinking water for three generations at a dose level of 100 mg/kg/day (20 mg nitrite-nitrogen/kg/day). No teratogenic effects or adverse effects on reproduction were detected in any generation.

Sleight and Atallah (1968) studied the effects of nitrite on reproduction and development in guinea pigs. Groups of 3-6 females were given water containing 0, 300, 1,100, 2,000, 3,000, 4,000, 5,000 or 10,000 ppm of KNO_2 for 100-240 days. Based on measured water intakes and body weights, this corresponded to average doses of 0, 18, 45, 154, 182, 192, 244 or 577 mg nitrite-nitrogen/kg/day. Reproductive performance was relatively normal at exposure levels up to 3,000 ppm, but was severely affected at exposure levels of 4,000 ppm or higher. No live births occurred in animals given 5,000 or 10,000 ppm. This study identifies a reproductive NOAEL of 182 and a LOAEL of 192 mg nitrite-nitrogen/kg/day.

Shuval and Gruener (1972, 1977) studied the effects of sodium nitrite on growth and postnatal survival in rats. Groups of 12 pregnant rats (strain "sabra") were given water containing 0, 2,000 or 3,000 mg/L sodium nitrite through the third week after parturition. This corresponded to doses of 0, 54 or 81 mg nitrite-nitrogen/kg/day. Birth weights were similar in all groups. After birth, pups of treated dams lagged in growth rate. At the end of 21 days, mean weight of the control group was 51.5 g compared to 29.5 g and

18.5 g in the low- and high-dose treated groups, respectively. Neonatal mortality during the lactational period was 6%, 30% and 53% in the control, low dose and high dose groups, respectively.

Globus and Samuel (1978) studied the effect of nitrite exposure during pregnancy on the fetal hematopoietic tissues in CD-1 mice. Groups of 36-39 mice were intubated with 0 or 16.7 mg/kg/day of $NaNO_2$ (3.4 mg nitrite-nitrogen/kg/day) from day 0 until sacrifice. The treated and control mice were divided into subgroups that were sacrificed on the 14th, 16th or 18th day of gestation. Analysis of fetal livers indicated that maternally administered sodium nitrite stimulated fetal hepatic erythropoiesis. After removal of viscera, the fetuses were fixed and stained for skeletal examination. Fetal mortality, fetal resorption, the mean number of offspring per litter, the mean weight per fetus, and the incidence of skeletal malformations were not significantly different from controls (P value not stated).

Hugot et al. (1980) performed a three-generation study in rats. Groups of 66 female animals were administered 0, 1,500 ppm or 3,000 ppm $NaNO_2$ in the diet. These levels are equivalent to 90 or 160 mg nitrite-nitrogen/kg/day. There were no effects on the percentage of matings resulting in pregnancy, the average of implanted embryos, the frequency of resorptions, the percentage of pregnancies resulting in the birth of live litters, the sex ratio or the percentage of born animals that survived 3 days. Birth weight was slightly lower in F_{1b} and F_{2b} pups in the group fed 3,000 ppm. After birth, the rate of weight gain by pups of the 1,500 ppm group and the 3,000 ppm group lagged behind the control group by 10% and 30%, respectively. This effect was most prominent in the F_{2a} and F_{2b} groups. In the treated groups, changes in various organ weights were observed in a high proportion of pups at weaning. These included increased liver, kidney and heart weights and decreased spleen and thymus weights. Histological studies were done on F_{2b} pups only. The main abnormalities observed in these weanlings were fatty degeneration of the liver and congestion of the spleen. The authors concluded that the effects of nitrite ingestion on fertility and gestation in the rat were very limited, but that the body and organ growth of the pups were affected between birth and weaning. This study identifies a LOAEL of 90 mg nitrite-nitrogen/kg/day.

Olsen et al. (1984) fed a diet containing potassium nitrite-treated meat to 70 male and 140 female Wistar rats. The nitrite content of the diet was reported to be 2, 4 or 94 mg/kg (corresponding to doses of 0.02, 0.04 or 0.9 mg nitrite-nitrogen/kg). Rats were fed this diet for 10 weeks prior to mating. No differences in pregnancy rate, litter size, mean pup weight or survival were observed between treated and control rats. No teratogenic effects were reported.

Vorhees et al. (1984) investigated the developmental toxicity of sodium nitrite in rats. Groups of males (numbers not specified) and females (22-32/dose group) were given food containing 0, 0.0125, 0.025 or 0.05% $NaNO_2$ for 14 days prior to breeding and during breeding (14 days). Exposure of females was continued during gestation (22 days) and lactation (21 days). After weaning, offspring were supplied $NaNO_2$ in the diet at the same level as their parents for 90-180 days. No effects were observed for a number of reproductive parameters, including number of females with sperm, percentage of females with sperm delivering, number of litters with more than 8 pups, gestational lengths, number of pups per litter and male/female ratio. No malformations were detected. However, $NaNO_2$ produced a statistically significant increase in preweaning mortality and a decrease in pup body weight in the middle and high dose groups. Pups were subjected to a number of neurobehavioral tests. Most tests were negative, but delays in swimming development were detected in the middle and high dose group during lactation. Decreased open field activity was also noted, but this was not dose dependent. The authors interpreted the results to reflect a delay in development rather than specific central nervous system damage. This study identifies a NOAEL of 0.0125% and LOAEL of 0.025% in food. This corresponds to doses of about 1.2 and 2.5 mg nitrite-nitrogen/kg/day to the dam.

Roth et al. (1987) supplied groups of 8-10 females Long-Evans hooded rats with water containing 0, 2,000 or 3,000 mg/L of $NaNO_2$ throughout gestation and lactation. Based on measured water intake rates, the average daily exposures were 44 or 60 mg nitrite-nitrogen/kg/day during gestation and 84 or 103 mg nitrite-nitrogen/kg/day during lactation. No evidence of maternal toxicity was described. No significant differences in litter size, pup weight or sex ratio were observed at birth between pups born to treated vs untreated dams. However, pups born to treated dams did not gain weight as well as controls and pup mortality at day 21 was increased in both dose groups. The affected pups were pale, weak and had distended abdomens. At day 9, pups from the low and high-dose groups were anemic, with hemoglobin levels of 6.70 g/dL and 6.13 g/dL respectively, compared to 9.85 g/dL in controls. The anemia became somewhat more severe by day 16. Blood smears revealed hypochromasia and anisocytosis in treated pups. Methemoglobin was not elevated in either treatment group compared to controls.

Roth et al. (1987) supplied groups of 5-8 female Long-Evans hooded rats (180-200 g) with drinking water containing 0, 500, 1,000 or 2,000 mg/L of $NaNO_2$ throughout gestation and lactation. This corresponds to doses of 0, 10, 20 or 40 mg nitrite-nitrogen/kg/day. Maternal weight gain during gestation did not differ between groups. Selected pups were sacrificed and blood samples collected 7, 9, 13, 16 and 20 days postpartum. Dose dependent decreases were noted in hemoglobin concentration, red blood cell count and mean corpuscular volume. Of these, mean corpuscular volume appeared to be the most sensitive indicator of effect. Statistically significant decreases were noted in the two highest dose groups on all days treated. This study identifies a NOAEL of 10 and a LOAEL of 20 mg nitrite-nitrogen/kg/day.

Roth et al. (1987) provided pregnant Long-Evans hood rats with water containing 0 or 2,000 mg $NaNO_2$/L during gestation. This corresponds to doses of 0 or 40 mg nitrite-nitrogen/kg/day. Following birth, the pups were separated from their maternal dams and were distributed to foster dams (10 pups/dam) as follows: control pups (mothers given tap water) to control dams (5 litters); exposed pups to control mothers (6 litters); control pups to exposed dams (5 litters); and exposed pups to exposed dams (5 litters). Selected pups were killed on days 7, 14 and 21 for hematological and histological examination. No significant differences were observed between exposed and control groups with respect to pup weight, litter size, sex ratio or postpartum mortality. Pups suckled by dams exposed to sodium nitrite in their drinking water weighed significantly less than pups suckled on dams drinking tap water. This effect was seen regardless of prenatal treatment received by the pups. Anemia was also observed in pups by day 14 suckled on dams exposed to nitrite-containing water. Red blood cell counts, mean corpuscular volume and hemoglobin were significantly reduced in these pups. These effects were not observed in pups exposed only during gestation. The authors concluded that post-natal exposure (via the milk during lactation) was more important in the anemic response than was exposure during gestation. This study identifies a LOAEL of 40 mg nitrite-nitrogen/kg/day, but does not identify a NOAEL.

Shiobara (1987) administered doses of 0, 20, 40, 80 or 120 mg/kg/day of $NaNO_2$ to pregnant mice (25/dose) on days 6-15 of gestation. This corresponds to doses of 0, 4, 8, 16 or 24 mg nitrite-nitrogen/kg/day. Fetuses were examined on day 17 for external and skeletal malformations. In the two highest dose groups, females gained less weight than normal, and the number of implants, living fetuses and litter size were all significantly decreased. No treatment-related teratogenic effects were detected. This study identifies a NOAEL of 8 and a LOAEL of 16 mg nitrite-nitrogen/kg/day.

Alavantic et al. (1988b) exposed groups of 5 male C3H X 101 mice to 0, 60 or 120 mg/kg/day sodium nitrite (0, 12 or 24 mg nitrite-nitrogen/kg/day) by stomach intubation for 3 days. Mice were sacrificed 11 and 17 days following treatment and 500 sperm cells per animal from the caudal epididymis were examined for abnormal morphology. The number of abnormal sperm heads were elevated in mice given 60 mg/kg/day sodium nitrite, although this elevation was not statistically significant. Sperm-head abnormalities were significantly elevated in mice receiving 120 mg/kg/day sodium nitrite. This study identifies a NOAEL of 60 mg/kg/day for sperm-head abnormalities from sodium nitrite exposure.

Alavantic et al. (1988a) investigated the effect of sodium nitrite on reproduction in mice. Male and female C3H X 101 mice (10-12 weeks old, 25/group) were dosed intragastrically with 0, 60 or 120 mg/kg/day $NaNO_2$ (0, 12 or 24 mg nitrite-nitrogen/kg/day) daily for 14 days prior to mating. Fertility of parent mice, litter size and sex ratio were observed and the number of heritable translocations and sperm-head abnormalities were assessed in both parent and offspring. Fertility and litter size were reduced in mice receiving 60 mg/kg/day, although the reduction was not statistically significant. A significant reduction in the number of fertile females was observed at 120 mg/kg/day. Cytogenic evaluation of the spermatocytes of parental mice revealed a significant elevation of sex chromosome univalency at 120 mg/kg/day $NaNO_2$ but not at 60 mg/kg/day. Sodium nitrite did not induce translocations in stem spermatogonia at diakinesis-metaphase I. Sperm-head abnormalities were significantly increased at both doses. Nitrite was not shown to induce heritable chromosomal translocations or sex chromosome univalency in the offspring of treated mice. No statistically significant increases in the number of sperm-head abnormalities were apparent in F_1 mice and no changes were observed in testes weight or body weight. This study identifies a LOAEL of 60 mg/kg/day $NaNO_2$ for the increased number of spermhead abnormalities seen in mice.

Roth and Smith (1988) studied the maternally mediated toxicity of nitrite in Long-Evans hooded rats (200 g) supplied with drinking water containing 0, 2,000 or 3,000 mg $NaNO_2$/L prior to and throughout gestation and lactation. This corresponds to doses of 0, 40 or 60 mg nitrite-nitrogen/kg/day. Pups from treated dams had reduced growth rates, increased rates of mortality, severe microcytic anemia, lipemia, fatty liver damage, decreased erythropoiesis of both spleen and bone marrow and decreased plasma and tissue iron. Treated dams also had significantly reduced plasma iron and reduced milk iron. Supplementation of the diet with iron reduced the severity of these effects. This study identifies a LOAEL of 40 mg nitrite-nitrogen/kg/day for maternally mediated iron deficiency anemia in rat pups.

Shimada (1989) investigated the teratogenic potential of nitrite in mice. Groups of 16-19 pregnant females were given drinking water containing 0, 100 or 1,000 mg/L[(a)] from days 7-18 of pregnancy. The average amount of $NaNO_2$ consumed was 0, 0.81 or 7.3 mg/mouse/day (about 0, 5.4 or 49 mg nitrite-nitrogen/kg/day). No detectable changes were noted for maternal weight gain, pregnancy rate, implants/litter, live fetuses/litters, percentage of dead or resorbed fetuses/litter, sex ratio, average fetal body weight, number of runt/litter, type or number of external or skeletal malformations, or chromosomal aberrations in fetal liver. This study identifies a developmental NOAEL of 49 mg nitrite-nitrogen/kg/day.

Nitrite plus Nitrosable Substrates

Ivankovic et al. (1973) investigated the teratogenic effect of oral ethylurea (100 mg/kg) and sodium nitrite (10 mg nitrite-nitrogen/kg) in pregnant rats. A total of 160 offspring were born. Four to six weeks after birth, 96 (60%) of the young animals developed hydrocephalus and died within 2 weeks. No other malformations were seen. Seventeen additional pregnant

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(a)The authors reported this a 0, 100 or 1,000 mg/mL. It is presumed that mg/L was intended.

Wistar rats were given the same treatment but with ascorbic acid (250 mg/kg) administered orally at the same time. A total of 113 offspring were reared after birth and none showed symptoms of hydrocephalus 1 year later.

Teramoto et al. (1980) administered ethylenethiourea (400 mg/kg) to pregnant mice in combination with 50, 100 or 200 mg/kg sodium nitrite (10.2, 20.3 or 40.6 mg/kg as nitrogen). When nitrite was given to mice immediately after their treatment with ethylenethiourea on day 6 or 9 of pregnancy, fetal survival was decreased. Various types of malformations were observed in the living fetuses from dams treated with the two higher doses of nitrite plus ethylenethiourea on days 6, 8 or 10 of pregnancy, but malformations were not observed in fetuses from dams treated on day 12. Teratogenicity was not observed when nitrite was given 2 hours after ethylenethiourea or when either chemical was administered alone.

D. Mutagenicity

Nitrate

The U.S. Food and Drug Administration (FDA 1972e) evaluated the genotoxic potential of $NaNO_3$ in a number of assays systems. The compound did not produce a measurable mutagenic response in Salmonella typhimurium or an increase in recombination frequency or Saccharomyces cerevisiae, either in vitro or in a host-mediated assay in mice. The compound did not produce chromosomal abnormalities in bone marrow cells from rats, but did result in a 2-fold increase in the number of acentric fragments in cultures of human embryonic lung tissue. No consistent dose-dependent effect was detected ~~in~~ a dominant lethal gene test in rats. Subsequent tests of KNO_3 in the dominant lethal mutation assay were also negative (FDA 1979a).

Ishidate et al. (1984) studied the effect of $NaNO_3$ on the reverse mutation rate in *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98, with or without activation by rat liver microsomal fractions (S-9). Chromosome aberration tests *in vitro* were carried out using a cultured Chinese hamster fibroblast (CHL) cell line without metabolic activation. Sodium nitrate did not induce a significant increase in the number of revertant colonies in the Salmonella/microsome assay, but did increase the frequency of chromosomal aberrations in CHL cells.

Luca et al. (1985) administered 2 gavage doses of sodium nitrate at 24-hour intervals to male Wistar rats (10-12 weeks old). The doses administered were 0, 78.5, 235.5, 706.6 or 2,120 mg $NaNO_3$/kg (0, 16, 47, 140 or 420 mg nitrate-nitrogen/kg). Rats were sacrificed 24 hours after the second dose was administered. Chromosomal aberrations (gaps, breaks, acentric fragments and exchanges) were examined in metaphase cells from bone marrow. No significant differences in the number of structurally abnormal chromosomes were seen in treated rats. When exposure was extended to 2 weeks, the percentage of aberrant metaphases was significantly increased in treated rats in an apparent dose-related fashion.

Luca et al. (1985) administered 2 gavage doses (at 24 hour intervals) of sodium nitrate to groups of 8 male Swiss mice (12-14 weeks old). The doses administered were 0, 78.5, 235.5, 706.6 or 2,120 mg $NaNO_3$/kg (0, 13, 39, 120 or 350 mg nitrate-nitrogen/kg). Mice were sacrificed 6 hours after the second dose for the evaluation of micronuclei or 24 hours after the second dose for examination of chromosome aberrations (gaps, breaks, acentric fragments and exchanges) in metaphase cells from bone marrow. The percent of cells with structurally abnormal chromosomes was not significantly affected by treatment except in the group of mice receiving 706.6 mg/kg $NaNO_3$. An effect was not seen in mice receiving the highest dose.

Alavantic et al. (1988b) studied the effect of $NaNO_3$ on the rate of unscheduled DNA synthesis (UDS) in spermatids in male mice. Groups of 5 animals (10-12 weeks old) were exposed to either 0, 600 or 1,200 mg/kg/day sodium nitrate by stomach intubation (0, 100 or 200 mg nitrate-nitrogen/kg/day). Mean levels of UDS measured by injection of ^3H-thymidine were not significantly affected at either dose level.

Nitrite

The U.S. Food and Drug Administration (FDA 1972f) evaluated the genotoxic potential of $NaNO_2$ in a number of assays systems. The compound did not produce a measurable mutagenic response in *Salmonella typhimurium* or an increase in recombination frequency in *Saccharomyces cerevisiae* in a host-mediated assay in mice, but did yield positive responses in both organisms *in vitro*. The compound did not produce chromosomal abnormalities in bone marrow cells from rats, but did result in a sharp increase in the number of acentric fragments in cultures of human embryonic lung tissue. No consistent dose-dependent effect was detected in a dominant lethal gene test in rats. Subsequent tests of KNO_2 in the dominant lethal mutation assay were also negative (FDA 1979b).

Couch and Friedman (1975) found no evidence of mutagenicity for sodium nitrite in a host-mediated assay in male ICR mice. Salmonella typhimurium were injected intraperitoneally immediately after gavage with 150 mg/kg sodium nitrite (30 mg/kg nitrite-nitrogen).

Kodama et al. (1976) studied the mutagenic effect of sodium nitrite on a cultured mouse mammary tumor cell line. Severe chromosomal aberrations were induced in 40% of the cells incubated with 3 mM and 80% at 10 mM $NaNO_2$. The frequency of 8-azaguanine resistant cells was increased in cultured incubated with concentrations greater than 1 mM $NaNO_3$.

Whong et al. (1979) injected Salmonella typhimurium intravenously into female mice and rats, followed by administration of $NaNO_2$ by gavage ten minutes later. The dose was 160 mg/kg (32 mg/kg nitrite-nitrogen) for mice and 150 mg/kg (30 mg/kg nitrite-nitrogen) for rats. No evidence of increased mutation frequency in the bacteria was detected.

Inui et al. (1980) reported a small increase (3.7 times control) in to 8-azaguanine resistance in Syrian hamster embryo cells cultured 24 hours after gavage of pregnant hamsters with 100 mg/kg sodium nitrite (20 mg/kg nitrite-nitrogen).

El-Nahas et al. (1984) studied the mutagenic potential of sodium nitrite on adult bone marrow and embryonic liver cells of the rat. Drinking water containing 1,250 mg sodium nitrite/L was provided to pregnant albino rats on days 5 through 18 of gestation. The authors estimated that this resulted in an average sodium nitrite dose of 42 mg nitrite-nitrogen/kg/day. Pregnant rats were sacrificed and 30 treated and 10 control embryonic livers retrieved. Bone marrow was obtained from the femurs of 6 adult control and 7 adult nitrite-treated rats. Adult bone marrow metaphase and metaphase cells from embryonic livers were examined for chromosomal aberrations. The number of chromosomal aberrations (breaks, centric fusions, dicentrics) were significantly increased in both adult bone marrow and embryonic liver. Chromosomal breaks were the most frequently seen, occurring in 7.3% of examined maternal bone marrow cells from treated rats compared to 3.3% of controls and in 6.4% of embryonic liver cells examined compared to 1.7% of controls.

Ishidate et al. (1984) reported that $NaNO_2$ produced a clear dose-dependent increase in mutation frequency in Salmonella typhimurium, with or without metabolic activation. Incubation of Chinese hamster fibroblast cells with $NaNO_2$ (250, 500 or 1,000 mg/L) in vitro resulted in a dose dependent increase in the incidence of chromosomal structural aberrations (11%, 16% and 22%, respectively).

Luca et al. (1987) administered sodium nitrite by gavage to groups of 6-8 male mice or rats at doses of 0, 1.72, 5.18, 15.55 or 46.66 mg/kg in distilled water (0.34, 1.0, 3.1 or 9.3 mg/kg nitrite-nitrogen). Animals were given 2 doses at 24 hour intervals and sacrificed 24 hours after the second nitrite dose. Bone marrow was examined for chromosomal aberrations (gaps, breaks, acentric fragments and exchanges) (200 cells scored per group) and micronuclei analysis performed. The percent of aberrant metaphases were significantly increased in treated mice and rats but there was no apparent dose-related trend. Micronucleated polychromatic erythrocytes (studied in mice only) were significantly increased in all but the high-dose group. The authors interpreted this to suggest that $NaNO_2$ was mutagenic at doses below its cytotoxic level.

Luca et al. (1987) administered 0, 1.72, 5.18, 15.55 or 46.66 mg/kg sodium nitrite in drinking water daily to groups of 6 male rabbits (30-32 weeks of age) for 3 months. Rabbits were sacrificed 24 hours after the last dose of sodium nitrite. Bone marrow was examined for chromosomal aberrations (gaps, breaks, acentric fragments and exchanges). Structural aberrations were significantly increased in the two low dose groups only.

Luca et al. (1987) incubated cultured monkey liver cells (BS-C-1) and HeLa cells with sodium nitrite in physiological saline at concentrations of 0.265 mg/mL or 0.530 mg/mL (0.053 or 0.106 mg nitrite-nitrogen/mL). Both doses resulted in significantly increased percentages of aberrant metaphases in the cultured cells, but no dose-related trend was observed.

Mukherjee et al. (1988) studied the effect of sodium nitrite on sister chromatid exchange (SCE) and micronuclei (MN) in mice. All animals were implanted with a paraffin-coated tablet containing 5-bromo-deoxyuridine. Sodium nitrite was administered by a single intraperitoneal injection of 25, 50, 75, 100 or 150 mg/kg (5, 10, 15, 20 or 30 mg nitrite-nitrogen/kg), and animals were sacrificed 24 or 48 hours later. All doses of nitrite resulted in statistically significant increases in the incidence of SCE and MN in bone marrow cells. Treatment with sorbic acid did not reduce these effects.

Alavantic et al. (1988b) studied the effect of sodium nitrite on the rate of unscheduled DNA synthesis (UDA) in spermatids in male mice. Groups of 5 animals (10-12 weeks old) were exposed to 0, 60 or 120 mg sodium nitrite/kg/day (12 or 24 mg nitrite-nitrogen/kg/day). Mice were sacrificed 17 days after treatment for evaluation of UDS. Mean levels of UDS (measured by injection of ^{3}H-thymidine) were not significantly different from controls at either dose level.

Nitrite plus substrate

Couch and Friedman (1975), Whong et al. (1979) and Inui et al. (1980) reported significant mutagenicity in stomach juice of animals following gavage with various nitrosatable substrates and sodium nitrite. These results are possibly due to the formation of mutagenic N-nitroso compounds in the stomachs of these rodents, rather than direct mutagenicity of sodium nitrate. Two other studies in mice detected mutagenic activity after gavage of nitrite and five other nitrosatable substrates using *Salmonella typhimurium* injected intraperitoneally or intravenously as genetic indicator (Braun et al. 1977, 1980).

E. Carcinogenicity

Effects of Nitrate Alone

Greenblatt and Mirvish (1972) supplied groups of 40 strain A mice with sodium nitrate in water at concentrations of 0 or 12,300 mg/L. This corresponds to doses of about 0 or 340 mg nitrate-nitrogen/kg/day. The animals were exposed for 25 weeks, and the lungs of the animals were examined histologically after an additional 13 weeks. The numbers of lung tumors in the treated and control mice were similar. No other tissues were examined. The treatment period may have been too short for carcinogenic effects to have occurred.

Lijinsky et al. (1973) administered 100 mg of sodium nitrate daily in drinking water to each of 15 male and 15 female MRC rats. This corresponded to a dose of 39 mg nitrite-nitrogen/kg/day. Administration was 5 days a week for 84 weeks followed by a 20-week observation period. An increase in pituitary tumors was observed in treated females (11/15 treated vs 3/15 controls). The authors stated "The high incidence of tumors of normal type in both experimental and control groups is unexpected and difficult to explain... Our groups of animals were small, and statistical analysis of the results could not be conclusive".

Maekawa et al. (1982) performed a 2-year bioassay of the carcinogenicity of sodium nitrate in F344 rats. Groups of 50 males and 50 females were administered 0, 25,000 or 50,000 ppm $NaNO_3$ in the diet. This corresponds to doses of 0, 200 or 400 mg nitrate-nitrogen/kg/day. Tumor incidence in all groups was close to 100%, but there were no significant differences in mean survival time, mean time to tumor, incidence of malignant tumors or incidence of any specific tumors.

Effect of Nitrite Alone

Garcia and Lijinsky (1973) administered sodium nitrite in drinking water at concentrations of 0 or 200 mg/L (corresponding to doses of about 0 or 5.5 mg nitrite-nitrogen/kg/day) to 15 MRC rats of each sex for 75 weeks. Animals were kept until death and subjected to complete pathological examination. There were a total of 19 tumor-bearing animals in the treated groups compared to nine in the controls. The only notable specific increase was seven pituitary tumors in treated rats compared to three in controls. This doubling was not statistically significant.

Sen et al. (1975) maintained groups of 20 male English short-hair guinea pigs with drinking water containing 0 or 800 mg/L sodium nitrite (160 mg nitrite-nitrogen/L) for 30 months. Although all guinea pigs were subjected to gross examination and histopathologic examination of nine major organs, only the observation regarding liver tumors were reported. There were no liver tumors in the unexposed or nitrite-exposed guinea pigs.

Shank and Newberne (1976) exposed 96 Sprague-Dawley rats of both sexes to sodium nitrite in the diet at a level of 0 or 1,000 ppm for 125 weeks. This corresponds to doses of 0 or 10 mg nitrite-nitrogen/kg/day. Exposed animals had a 27% incidence of tumors of the lymphoreticular system. This was compared to a 6% incidence in 156 untreated control rats. Significant increases in tumor incidence were not observed in other tissues. No tumors of the lymphoreticular system or any other carcinogenic effect were found in Syrian golden hamsters treated similarly.

In a follow-up study, Newberne (1978, 1979) administered sodium nitrite to groups of 68 Sprague-Dawley rats of each sex in water or in various diets from the time of conception through their natural life span. Doses ranged from 12.5 to 200 mg/kg/day (4-60 mg nitrite-nitrogen/kg/day). The rats survived well. The author observed the usual spectrum of neoplastic and non-neoplastic diseases associated with old rats. The tissue changes of most interest were observed in the spleen, lymph nodes and other components of the lymphoreticular tissues. The spleen exhibited a lesion described to as immunoblastic cell proliferation. When spleen and non-spleen lymphomas were combined, there were suggestions of a nitrite effect but there was no convincing dose-response relationship. Combining all nitrite-treated groups and all control groups, the incidence rates for lymphomas were 8.5% and 12.5% in control and nitrite-treated groups, respectively. Overall, the incidence rates of lymphoreticular neoplasia were 15.3% in untreated and 23.7% in nitrite-treated animals. Newberne's histopathologic assessment of the tissues indicated that, by considering all the groups receiving sodium nitrite together, there was a statistically significant excess of lymphoid tumors ($P<0.01$ based on chi-square analysis). This was reflected especially in the groups receiving sodium nitrite in drinking water.

After Newberne's report was submitted, a Government Interagency Working Group on Nitrite Research reviewed a sample of histological slides from the study and decided that there was sufficient difference of opinion in the diagnoses to warrant a further evaluation of the histopathological findings. The Universities Associated for Research and Education in Pathology (UAREP), a nonprofit consortium of 15 universities organized to carry out educational and research activities in pathology, was selected by the FDA to review the slides (FDA 1980a). A Joint Committee of Experts was established by the UAREP to perform this review. The committee members diagnosed fewer lymphomas than Newberne had reported. The disparity between the two series of diagnoses involved the differentiation of lymphomas from extramedullary hematopoiesis, plasmacytosis or histiocytic sarcoma. Furthermore, the committee did not interpret the lesions in the spleen and lymph nodes as immunoblastic hyperplasia as did Newberne. These lesions were interpreted predominantly as extramedullary hematopoiesis and plasmacytosis in the spleen and plasmacytosis or reactive lymphoid hyperplasia in lymph nodes.

In its final report to the FDA, the Government Interagency Working Group summarized the UAREP committee's findings and concluded that most of the lymphoma diagnoses originally reported were not confirmed. The UAREP pathologists diagnosed fewer lesions as lymphoma, with a resulting reduction of incidence to approximately 1% among treated and control groups. This rate of lymphoma incidence is similar to that usually seen spontaneously in Sprague-Dawley rats. UAREP pathologists did report a greater than 1% incidence of other types of tumors, including histiocytic sarcomas. However, no demonstration could be found that the increased incidences of these tumors were induced by the ingestion of sodium nitrite (FDA 1930b).

Taylor and Lijinsky (1975) and Lijinsky and Taylor (1977) provided drinking water containing 2,000 mg/L sodium nitrite (400 mg nitrite-nitrogen/L) to groups of 27 male and 30 female Sprague-Dawley rats 5 days/week for 104 weeks. Untreated controls were not maintained, but based on complete gross and microscopic pathologic examination of the nitrite-exposed group, Lijinsky and Taylor (1977) concluded that the identity and incidence of tumors observed were those expected in aged rats of this strain.

Inai et al. (1979) supplied sodium nitrite at concentrations of 1,250, 2,500 or 5,000 mg/L in drinking water (about 42, 83 or 167 mg nitrite-nitrogen/kg/day) to groups of 50 male and 50 female ICR mice for 18 months. No tumors attributable to nitrite treatment were observed in histopathological examinations of major organs.

Anderson et al. (1979) provided drinking water containing 14 mM sodium nitrite (200 mg nitrite-nitrogen/L) 4 days/week to 20 female and 17 male weanling Swiss mice for 8 months. Another group of 16 female and 21 male mice served as untreated controls. The mice were observed until spontaneous death, at which time they were subjected to necropsy and histopathological examination of all gross lesions. There was no evidence of a carcinogenic effect of exposure to nitrite.

Mirvish et al. (1980) reported that papillomas of the forestomach developed in 8 of 45 (18%) MRC Wistar rats (both sexes) administered an aqueous solution containing 3,000 mg/L of sodium nitrite (57.7 mg/kg/day as nitrogen) 5 days/week for life. Two of 91 untreated rats (2%) developed these tumors ($P<0.002$). These rats were fed Wayne Lab-Blox which contains fish meal, a source of nitrosatable amines, so this effect might have been due to formation of nitrosamines rather than nitrite per se (see below).

Pearson et al. (1980) administered drinking water containing 0 or 1,000 mg/L sodium nitrite (200 mg nitrite-nitrogen/L) to groups of 6 weanling mice (strain and sex not specified), maintained on a complete laboratory chow diet for 12 months. Survivors were killed at 12 months and subjected to gross examination and histopathologic examination of the liver, lungs, heart, spleen and stomach. This experiment was repeated 3 times over the next 3 years, using 10 mice per group. Thus, a total of 36 mice/group was examined. The tumor incidence in the nitrite-treated group was not significantly different from that in the nonexposed group.

Maekawa et al. (1982) supplied groups of 50 male and 50 female F344 rats with water containing 0, 1,250 or 2,500 mg/L of $NaNO_2$. This corresponds to doses of about 0, 2.5 or 5 mg nitrite-nitrogen/kg/day. Exposure was for 2 years. Tumor incidence in all groups was close to 100%, but there were no significant differences in mean survival time, mean time to tumor, increase of malignant tumors or increase of any specific tumor.

Lijinsky et al. (1983) provided two groups of 24 male and two groups of 24 female F344 rats (7-8 weeks old) with free access to food containing 2,000 ppm sodium nitrite (20 mg/kg/day as nitrite-nitrogen). One group of 24 females and one group of 24 males were given access to 2,000 mg/L sodium nitrite in their drinking water (56 mg/kg/day nitrite nitrogen), 5 days per week for 2 years. Untreated controls were included for each sex. Survival rates of treated rats were not significantly different from controls. Female rats exposed to nitrite in food had significantly higher incidences of hepatocellular neoplasms (27/48 vs 8/48, $P=0.0001$), although the number of liver carcinomas by themselves was not statistically different. No significant differences in the incidence of liver neoplasms were seen in males. The incidence of mononuclear cell leukemia was significantly reduced in nitrite-fed rats (males and females) and in female rats receiving nitrite in their drinking water.

In a two-generation carcinogenicity study, Olsen et al. (1984) fed a diet containing potassium nitrite-treated meat to 70 male and 140 female Wistar rats. The resultant nitrite content of the diet was reported to be 2, 4 or 94 mg/kg. This corresponds to doses of 0.02, 0.04 or 0.9 mg nitrite-nitrogen/kg/day. Rats were fed this diet for 10 weeks prior to mating. Male and female offspring (60-100/group) were continued on the same diet after weaning. At 128 weeks, all surviving F_1 rats were sacrificed and histological examination was conducted on an extensive group of tissues. The number of malignant tumors found in treated rats was not significantly greater than in controls, but there was a trend for increased dermal squamous cell carcinomas in males. A statistically significant increase in males with kidney adenomas was observed, but this could have been by chance. The doses used in this study are too low to draw reliable conclusions about the effects of nitrite on cancer incidence.

Ernst et al. (1987) supplied groups of 15 male and 15 female Syrian hamsters with food containing sodium nitrite at 0 or 2,000 ppm (400 mg nitrite-nitrogen/kg food), 5 days/week for their lifetimes. At death, the hamsters were subjected to a comprehensive gross and histopathologic examination. There were no significant differences between untreated and nitrite-exposed groups in the incidence of tumors.

Grant and Butler (1989) studied the carcinogenic effect of lifetime exposure of rats to nitrite. Groups of 50 males were given diets containing 0, 0.2% or 0.5% $NaNO_2$ for 115 weeks. This corresponds to average doses of 0, 20 or 50 mg nitrite-nitrogen/kg/day. Thorough histological examinations revealed no statistically significant increase in tumor incidence in any tissue. Significant decreases in tumor incidence were noted for total lymphomas, mononuclear cell leukemia and testicular interstitial cell tumors.

Lijinsky and Kovatch (1989) provided drinking water containing 0 or 0.2% sodium nitrite to F344 rats (8-weeks old, 19-24/group), 5 days per week for at least 2 years. This corresponds to doses of 0 or 40 mg nitrite-nitrogen/kg/day. No adverse effects (including increased mortality) were seen in treated rats, and the number and type of neoplasms were not significantly affected by treatment. The incidence of mononuclear cell leukemia was significantly decreased.

Effect of Nitrite Administered with Nitrosable Substrates

Greenblatt et al. (1971) provided groups of 40 male and 40 female mice with water containing at 1,000 mg/L of $NaNO_2$ (5 days/week) with or without piperazine (6,250 mg/kg), morpholine (6,330 mg/kg) or methylaniline (1,950 mg/kg) in the diet. Exposure was terminated after 28 weeks. Animals were sacrificed after an additional 12 weeks, and the lungs were examined for the presence of tumors. Exposure to $NaNO_2$ in combination with any one of the amines resulted in a statistically significant increase in both the percentage of animals with tumors and the number of tumors per tumor-bearing mouse. This was more pronounced in males than females. Exposure to $NaNO_2$ or the amines alone did not cause increase in the incidence of tumors. The authors concluded that tumor induction was due to *in vivo* formation of carcinogenic nitrosamines.

Mirvish et al. (1972) supplied groups of 31-74 Swiss mice with food containing methylurea or ethylurea (5,360 ppm or 6,360 ppm, respectively) along with water containing 0 or 1,000 mg/L of $NaNO_2$. This corresponds to a dose of 0 or 30 mg nitrite-nitrogen/kg/day. Animals were exposed for 20 weeks and sacrificed 12 weeks later. Exposure to alkylurea plus nitrite resulted in statistically significant increases in the percentage of mice with lung adenomas and in the number of adenomas per mouse. Neither alkylurea nor $NaNO_2$ alone had a significant effect on tumor incidence.

Greenblatt and Mirvish (1972) supplied groups of 40 male mice with diets containing 0 or 6,250 ppm of piperazine, along with water containing 0, 50, 250, 500, 1,000 or 2,000 mg/L $NaNO_2$. This corresponds to a dose of about 0, 1.5, 7.5, 15, 30 or 60 mg nitrite-nitrogen/kg/day. Exposure was for 20 weeks. The animals were sacrificed and examined for lung tumors 10 weeks later. There was a clear dose-response trend toward increased numbers of mice with adenomas and adenomas per adenoma-bearing mouse ($P<0.001$ for all doses except 50 mg/L). Exposure to piperazine alone (6,250 ppm) or nitrite alone (2,000 mg/L) did not result in a significant increase in tumors.

Greenblatt et al. (1973) exposed groups of rats (15 males plus 15 females/group) to $NaNO_2$ and one of three secondary amino acids (proline, hydroxyproline, arginine) in drinking water. Doses were 10 mg nitrite-nitrogen/kg/day plus 25 mg/kg/day of amino acid. After 122 weeks, animals were sacrificed and subjected to histological examination for tumors. There were no detectable increases in the incidence of any tumors in any exposure group. The authors concluded that the nitrosoamino acids presumably formed by this treatment were not carcinogenic.

Taylor and Lijinsky (1975) administered 0.2% sodium nitrite (27 mg nitrite-nitrogen/kg/day) and 0.2% heptamethyleneimine (133 mg/kg/day) in drinking water to 15 male and 15 female Sprague-Dawley rats fed Purina lab chow. Rats were 8- to 10-weeks-old at the start and were treated for 28 weeks and observed until death. This treatment induced tumors of the oropharynx, tongue, forestomach or esophagus in 27 of 30 rats. No tumors were observed in animals exposed to nitrite alone or heptamethyleneimine alone.

Mirvish et al. (1976) tested the effect of sodium ascorbate on the oncogenic response induced by a combination of sodium nitrite and morpholine in groups of 40 eight-week-old male MRC-Wistar rats. In this study, three groups were used. Group I was treated with 3,000 mg/L of sodium nitrite in drinking water (81 mg nitrite-nitrogen/kg/day) and 500 mg/kg/day morpholine in food. Group II was administered the same treatment plus 1,135 mg/kg/day sodium ascorbate in food. The control group was kept on normal diet and plain water. Liver tumors appeared in 65% of group I, 49% of group II and none of the controls. Forestomach tumors appeared in none of group I, 54% of group II and 4% of untreated animals. Thus, in this study, a marked increase in forestomach tumors was observed in animals that received sodium ascorbate.

Lijinsky and Rueber (1980) administered 0 or 0.2% sodium nitrite and 0 or 0.1% disulfiram in a powdered diet to 40 Fischer 344 rats. This corresponds to doses of 0 or 20 mg nitrite-nitrogen/kg/day. Rats were 8- to 9-weeks-old at the start of treatment which continued for 78 weeks followed by observation until natural death. Exposure to nitrite plus disulfiram induced tumors of the stomach, esophagus, tongue and nasal cavity in 10 of 20 male and 12 of 20 female rats. None of these tumors were found in rats administered nitrate or disulfiram alone. The nitrosation product of disulfiram is nitrosodiethylamine.

Bergman and Wahlin (1981) supplied groups of 10 Syrian hamsters with water containing 0 or 0.1% aminopyrine and 0 or 0.1% $NaNO_2$. This corresponds to a dose of 0 or 17 mg nitrite-nitrogen/kg/day. After 20 weeks, animals were sacrificed and examined for tumors of the liver, gallbladder, lungs and kidneys. No tumors were observed in animals supplied with aminopyrine or nitrite alone. Nearly all animals in the group exposed to both aminopyrine and nitrite showed multiple foci of bile duct adenomas, and 6/10 developed cholangiocarcinoma of the liver. No tumors were detected in other tissues.

Lijinsky (1984) exposed groups of 20-24 male rats to 0.2% $NaNO_2$ in drinking water or food for their lifetimes. This corresponds to doses of about 20-40 mg nitrite-nitrogen/kg/day. Animals were also exposed to one of four nitrosable amines (allantoin, dimethyldodecylamine-N-oxide, diphenhydramine and chlorpheniramine) in the diet or in drinking water (0.1%-0.2%). None of the amines along resulted in increased incidence of liver neoplasms, but $NaNO_2$ plus diphenhydramine, chlorpheniramine or dimethyldodecylamine oxide all resulted in significant increases in liver tumors (hepatocellular carcinomas and neoplastic modules). A marginal increase was noted for allantoin plus $NaNO_2$.

Thamavit et al. (1988) administered aminopyrine (0 or 1,000 mg/L) and $NaNO_2$ (0 or 1,000 mg/L) in drinking water to groups of 14-18 male Syrian hamsters for 8-10 weeks. This corresponds to doses of 0 or 20 mg nitrite-nitrogen/kg/day. After an additional period of 2-20 weeks, the animals were sacrificed and subjected to histological examination for liver neoplasms. No tumors or nodules were detected in control animals or in animals exposed to nitrate or aminopyrine alone. In animals exposed to both, 3/17 animals had liver carcinomas, 7/17 animals has cholangiofibrosis and 2/17 had hepatocellular nodules. The incidence of these tumors was further increased by prior dosing with liver parasites that caused inflammatory and proliferative responses in the liver.

Mokhtar et al. (1988) exposed groups of 80 male mice to water containing 2,000 mg/L $NaNO_2$ and 1,000 ppm dibutylamine. This corresponds to a dose of 40 mg nitrite-nitrogen/kg/day. Histopathological examinations of liver were performed on groups of 15-20 after 3, 6, 9 or 12 months of exposure. Exposed animals developed hepatic dysplasia and necrosis within 3-6 months, and this became more pronounced with continued exposure. Hepatomas were found in 3/20 mice exposed to 9 months and 1/15 mice exposed to 12 months. No liver tumors were detected in control mice (exposed to neither $NaNO_2$ nor dibutylamine) of the same age. This tumorigenic response was diminished by concurrent feeding of soybean diet or ascorbic acid.

Yamamoto et al. (1989) administered bis(2-hydroxylpropyl)amine (BHPA) in the diet to groups to 20 male rats, along with drinking water containing 0, 1,500 or 3,000 mg/L of $NaNO_2$. This corresponds to doses of 0, 30 or 60 mg nitrite-nitrogen/kg/day. Exposure was for 94 weeks. Histological examination of tissues revealed an increased incidence of tumors of the nasal cavity (14/19), lung (11/19) and esophagus (2/19) in the high dose group. In the low dose group, increased tumors were seen only in lung (3/19). No tumors were seen in any of these tissues in control animals or in animals exposed to BHPA or $NaNO_2$ alone. The authors attributed this effect to formation of N-nitrosobis(2-hydroxypropyl)amine (BHP) in vivo. This was supported by the detection of BHP in the urine of animals exposed to BHPA and $NaNO_3$, but not in other groups.

Studies on In Vivo Nitrosamine Formation

Inui et al. (1980) administered 25, 50 or 100 mg/kg aminopyrine and 25, 50 or 100 mg/kg sodium nitrite (5, 10 or 20 mg nitrite-nitrogen/kg) to adult golden hamsters by gavage. Stomach contents were then examined for the presence of nitrosamines. A dose-dependent increase in N-nitrosodimethylamine (0.1-1.49 mg/animal) was measured within 30 minutes of the administration of aminopyrine and sodium nitrite. The concentration of dimethylnitrosamine in the stomach was much lower (0.01-0.04 mg/animal) in hamsters treated with sodium nitrite alone, and was not detectable in animals treated with aminopyrine alone or in untreated controls.

Massey et al. (1988) studied endogenous nitroso compound formation in normal and germ-free rats supplied with nitrite in drinking water (0, 50 or 500 mg NO_2/L). Nitroso compounds could not be detected in blood or tissues of any group, but were detected in the stomach and intestines of conventional rats at levels that increased as a function of nitrite concentration in water. Nitroso compounds were detected in stomach and intestines of germ-free rats, but at levels lower than conventional animals. These data were interpreted by the authors to indicate that enteric bacteria converted dietary or endogenous substrates to nitroso compounds, and that increased levels of nitrite in water increased the amount of nitroso compound formation.

F. Summary

The toxic effects of nitrate are closely related to its conversion to nitrite by bacteria in the alimentary tract. Thus, the toxicity of nitrate depends not only on dose, but also on the level and type of bacteria present. Consequently, dose-response relationships are quite variable between different animal species. For example, ruminants are highly sensitive, while dogs have low sensitivity. Because of this species variability, animal data should be extrapolated to humans with caution.

The principal health effect from exposure to nitrate or nitrite is methemoglobinemia. This results when nitrite oxidizes the Fe^{+2} form of iron in hemoglobin to the Fe^{+3} state. In rats, acute oral LD_{50} values for $NaNO_3$ range from 1,000 to 2,000 mg/kg. These are close to LD_{50} values for NaCl, suggesting that acute lethality from $NaNO_3$ is caused mainly by nonspecific electrolyte imbalance. Chronic exposure to doses up to 69 mg nitrate-nitrogen/kg/day did not produce methemoglobinemia in rats, but doses of 50 mg/kg/day or higher have been noted to produce mild histological changes in liver or spleen. High doses may also interfere with thyroid function.

Nitrite is substantially more toxic to rodents than nitrate. Acute oral LD_{50} values range from 40 to 120 mg/kg (20-40 mg nitrite-nitrogen/kg/day), while doses of 20 mg nitrite-nitrogen/kg/day can produce methemoglobinemia and other related histological effects. High doses of nitrite act on vascular smooth muscle to produce vasodilation and hypotension, and may also cause changes in coronary blood vessels.

Numerous studies on reproductive function, teratogenicity and fetal development in several animal species have not revealed evidence of adverse effects from nitrate or nitrite except at relatively high dose levels. Such effects are likely to be secondary to maternal or fetal methemoglobinemia and related toxicity. Exposure of males to high doses may result in cytogenetic abnormalities in spermatocytes and morphological abnormalities in sperm, but this did not result in any apparent effect on reproduction or offspring. Ingestion of 20-100 mg nitrite-nitrogen/kg/day by dams during lactation can cause iron deficiency in milk and moderate to severe anemia and other post-natal effects in sucklings.

Most investigations on the mutagenicity of nitrate and nitrite have been negative. However, many studies have reported that both nitrate and nitrite lead to a variety of chromosomal aberrations, both in cultured cells *in vitro* and in animals exposed *in vivo*.

The carcinogenic potential of nitrate has not been extensively studied in animals, but several investigations have not revealed any evidence of increased tumor incidence. The carcinogenic potential of nitrite has been much more thoroughly studied. One early study was interpreted as providing evidence of increased tumors of the lymphoreticular system in rats, but re-evaluation of the histological slides from this study led to the conclusion that the lesions were not neoplastic. Numerous other studies of nitrite carcinogenicity in animals have also been negative. However, when nitrite is given to animals along with high levels of nitrosable amines, clear increases in tumor incidence are often observed in a variety of tissues. This tumorigenic response is probably a result of the formation of carcinogenic nitrosamines by reaction of nitrite with the nitrosable substrates in the stomach.

VI. HEALTH EFFECTS IN HUMANS

A. Methemoglobinemia

The principal adverse health effect associated with acute exposure of humans to nitrate or nitrite is methemoglobinemia. Low levels of methemoglobin occur in normal individuals, with typical values usually ranging from 0.5% to 3.0% (Winton et al. 1971, ECETOC 1988). However, due to the large excess capacity of blood to carry oxygen, levels of methemoglobin up to around 10% are not associated with any significant clinical signs (Walton 1951, Winton et al. 1971, ECETOC 1988). Concentrations above 10% may cause a bluish-color to skin and lips (cyanosis), while values above 25% lead to weakness, rapid pulse and tachypnea (Jones et al. 1973). Death may occur if methemoglobin values exceed 50%-60%.

Nitrite converts hemoglobin to methemoglobin by oxidizing the Fe^{+2} ion in heme to the Fe^{+3} state. Nitrate does not carry out this reaction unless it is first converted to nitrite. Conversion of nitrate to nitrite is mostly mediated by bacteria in the gastrointestinal system (mouth, stomach). Consequently, the risk of methemoglobinemia from ingestion of nitrate depends not only on the dose of nitrate, but also on the number and type of enteric bacteria. In adults, available data suggest about 5% of a dose of nitrate is reduced to nitrite (ECETOC 1988). Infants are more susceptible to nitrate toxicity than adults, since the high pH of the infant gastrointestinal system favors the growth of nitrate-reducing bacteria. In particular, infants exposed to bacteriologically-contaminated waters are susceptible to methemoglobinemia, since the ingested bacteria are likely to flourish in the stomach (ECETOC 1988).

1. Methemoglobinemia in Infants

Cornblath and Hartmann (1948) supplied water containing 1,000 mg NO_3/L to four infants (ages 11 days to 11 months) for 2-18 days. The amount administered yielded doses of 50 mg NO_3/kg/day (11 mg nitrate-nitrogen/kg/day). This intake resulted in a methemoglobin concentration of 5.3%, but there was no evidence of cyanosis. Four additional infants (2 days to 6 months of age) were given 100 mg NO_3/kg/day (23 mg nitrate-nitrogen/kg/day) for periods of 6-9 days. No cyanosis was evident in these infants, and the highest methemoglobin concentration observed was 7.5% (at 8 days in a 10-day-old infant). This study identifies a NOAEL of 23 mg nitrate-nitrogen/kg/day, which corresponds to 140 mg nitrate-nitrogen/L in water (assuming intake of 0.16 L/kg/day by an infant).

Donahoe (1949) reported five cases of moderate to severe cyanosis in infants in South Dakota. In four of the five cases, the water used to feed the infants was from shallow wells and was shown to be heavily contaminated with bacteria. Nitrate levels were measured in two cases, with values of 50 and 177 mg/L (12 and 41 mg nitrate-nitrogen/L), respectively. This corresponds to doses of 8 and 28 mg nitrate-nitrogen/kg/day.

Bosch et al. (1950) evaluated 139 cases of methemoglobinemia reported by physicians in Minnesota. All of the cases were in young children, with 90%

occurring in infants less than 2 months of age. A study of the nitrate concentration of the wells (a total of 129) used to supply water to the children with methemoglobinemia was performed. None of the wells contained less than 10 mg/L nitrate-nitrogen. Two wells (1.5%) contained 10-20 mg/L, although the diagnosis of methemoglobinemia was considered questionable in both these cases. There were 25 wells (19%) that contained 21-50 mg/L, 53 (41%) that contained 51-100 mg/L, and 49 (38%) that contained more than 100 mg/L nitrate-nitrogen. Nearly all the wells were shallow dug wells with inadequate protection from surface contamination. Coliform organisms were detected in 45 of 51 samples (88%) of samples tested for bacterial contamination.

Walton (1951) presented the results of a survey conducted by the American Public Health Association on morbidity and mortality among infants due to methemoglobinemia resulting from the ingestion of nitrate contaminated water. The survey was conducted by means of a questionnaire sent to all 50 states. The results of the survey revealed over 278 cases of infant methemoglobinemia, including 39 deaths, which were shown to be "definitely associated with nitrate-contaminated water." These occurred in 14 different states, located mostly in the north-central portion of the U.S. Of 214 cases for which quantitative data were available on nitrate levels in water, none occurred in infants consuming water containing less than 10 mg nitrate-nitrogen/L. There were 5 cases (2%) in infants exposed to 11-20 mg nitrate-nitrogen/L, 36 cases (17%) in infants exposed to 21-50 mg/L and 173 (81%) in infants exposed to more than 50 mg/L. Data on levels of bacteriological contamination, incidence of gastrointestinal disease, nitrite levels or actual methemoglobin levels were not reported.

Ewing and Mayon-White (1951) reported two cases of severe methemoglobinemia in infants. In the first, the infant ingested water from a shallow well that was "heavily polluted" with bacteria and contained 200 mg/L of nitrite (46 mg nitrate-nitrogen/L, equivalent to a dose of 7.4 mg nitrate-nitrogen/kg/day). This infant died. In the second case, the infant ingested water from a shallow well heavily contaminated with coliform organisms and containing 95 mg/L of nitrite (22 mg nitrate-nitrogen/L, equivalent to 3.5 mg nitrate-nitrogen/kg/day). This infant recovered after a transfusion.

Simon et al. (1964) reported the results of a survey of infant methemoglobinemia associated with nitrate-contaminated water occurring between 1956 to 1964 in the Federal Republic of Germany. A total of 745 cases were located. For 306 of these cases, data were available on infant age and frequency of diarrhea (an indication of bacteriological colonization). These data indicated that 97.7% of the cases were 3-months-old or less and 53% had diarrhea. Data were available on the nitrate content of the drinking water for 249 of the 745 methemoglobinemia cases. In 83.8% of these cases, the nitrate content of the drinking water was >100 mg/L (equivalent to 23 mg/L nitrate-nitrogen); in 11.8% of the cases it was 50-100 mg/L (equivalent to 11-23 mg/L nitrate-nitrogen); and in 4.4% of the cases it was <50 mg/L (equivalent to 11 mg/L nitrate-nitrogen). Only three cases of methemoglobinemia (1.3%) were associated with water containing <20 mg/L nitrate (equivalent to 4.5 mg/L nitrate-nitrogen). The authors indicated that in each of these three cases, spinach and spinach juice were significant sources of nitrate and nitrite, and in one case nitrite was known to be present in the water (concentration not reported). Therefore, the total dose of nitrate plus nitrite in these cases was greater than predicted from just the water intake.

Simon et al. (1964) measured methemoglobin levels in infants from three towns in Germany. There were 89 infants receiving nitrate-free water, 38 infants receiving water containing 50-100 mg/L nitrate (11-23 mg nitrate-nitrogen/L) and 25 infants receiving water containing greater than 100 mg/L nitrate (>23 mg nitrate-nitrogen/L). A dose-response relationship between nitrate concentration in the water and methemoglobin concentration was apparent only in infants less than 3 months of age. In these infants, mean methemoglobin level was 1% in the infants receiving nitrate-free water, 1.3% in infants receiving 50-100 mg nitrate/L and 2.9% in the infants receiving water containing nitrate in excess of 100 mg/L. Mean methemoglobin levels in infants greater than 3 months of age were 0.8% (0-100 mg nitrate/L) or 0.7% (>100 mg nitrate/L). No clinical signs of methemoglobinemia were detected. Based upon their finding and reviews, the authors conclude that 100 mg/L nitrate (23 mg nitrate-nitrogen/L) represents a safe level in water which is free of bacteria or other contaminants.

Toussaint and Selenka (1970) supplied 34 healthy infants (under 3 months of age) with formula prepared with water containing 150 mg NO_3/L (34 mg nitrate-nitrogen/L) for 10 days. Average methemoglobinemia levels rose from about 1% to about 2%-3% within 1-2 days, and then tended to stay steady for up to 10 days. No clinical signs of methemoglobinemia were reported.

Winton et al. (1971) studied a group of 111 infants who ingested varying levels of nitrate. Sixty-three received a nitrate dose of less than 1 mg/kg/day (equivalent to 0.23 mg/kg/day nitrate-nitrogen), 23 were exposed to 1-4.9 mg/kg/day (equivalent to 0.23-1.1 mg/kg/day nitrate-nitrogen), 20 were exposed to 5.0-9.9 mg/kg/day (equivalent to 1.1-2.2 mg/kg/day nitrate-nitrogen) and 5 infants were exposed to 10-15.5 mg/kg/day (equivalent to 2.3-3.6 mg/kg/day nitrate-nitrogen). Only three infants appeared to have methemoglobin levels above normal (0%-2.9%) and they were the youngest of the five who had received more than 10 mg/kg/day. The highest methemoglobin level (5.3%) was found in a 30-day-old baby who had received 15.5 mg/kg/day. Assuming that a 4-kg infant consumes 0.64 liters of formula per day (Davidson et al. 1975), these levels are equivalent to drinking water concentrations of approximately 14-22 mg nitrate-nitrogen/L.

Shearer et al. (1972) examined the association between age and methemoglobinemia in infants in south-central California. A total of 487 examinations were conducted on 256 infants over a period of 1 year. Approximately 26% of infants aged 0-3 months had methemoglobin levels >3%, while only 1% of infants older than 3 months had methemoglobin levels above this level. These results indicate that young infants (up to 3 months of age) were more likely to have elevated methemoglobin levels than older infants. No clear association between nitrate intake in the diet and methemoglobin level was detected.

Shuval and Gruener (1972) studied the association between drinking water nitrate and methemoglobinemia in infants in Israel. The study population consisted of 1,702 infants from an area in which the mean drinking water nitrate concentration was 70 mg/L (equivalent to 16 mg/L nitrate-nitrogen). The control population consisted of 758 infants from an area with a water supply containing 5 mg/L nitrate (equivalent to 1.1 mg/L nitrate-nitrogen). There were essentially no differences in methemoglobin levels between the two populations. The average level was 1.01% in the study area compared to 1.11% in the control area. In both areas, average methemoglobin levels tended to decrease as a function of age, from an average of about 1.33% in 0-2 month old infants to 0.98% in infants 91 days or older. Young infants (1-90 days) with diarrhea tended to have a somewhat higher average methemoglobin value (1.56%) than average, but this difference was not apparent in older infants.

Jones et al. (1973) reported a case involving the situation surrounding a previously healthy 10-week-old female baby who was admitted to the hospital with "striking" cyanosis. Blood methemoglobin was 47.6%. Investigation revealed that the child had been given one bottle of well water containing 110 mg/L of nitrate (25 mg nitrate-nitrogen/L) just prior to the acute methemoglobinemia. The infant was subsequently fully recovered and was discharged from the hospital within 1 week.

Gruener and Toeplitz (1975) fed formula containing varying levels of nitrate to 104 infants for 5 days. On the first day of the study, all infants were given formula made with water containing 15 mg/L nitrate (equivalent to 3.4 mg/L nitrate-nitrogen). For the next 3 days they were fed formula mixed with water containing 108 mg/L nitrate (equivalent to 24 mg/L nitrate-nitrogen). On the last day of the study the infants again received formula prepared with water containing 15 mg/L nitrate. There was a slight but significant rise in mean methemoglobin levels after the first day of exposure, although three individuals had especially high methemoglobin levels (6.9, 13.9 and 15.9%). There was a drop in mean methemoglobin levels to almost normal on the 3rd day. These levels remained constant on the 4th day and decreased further on the final day of the study, suggesting some adaptation.

Wurkert (1978) measured methemoglobin levels in 96 infants residing in various locations across Germany (Rheinhessen) and also sampled and measured nitrate levels in the drinking water. The majority (82%) of the infants were under the age of 3 months. The methemoglobin concentrations in the study population ranged from 0.33% to 14.15% with a mean of 2.30%. A significant statistical correlation was reported between the concentration of nitrate in the drinking water and methemoglobin concentration. Infants consuming drinking water with a nitrate concentration between 0-11 mg nitrate-nitrogen/L (0-50 mg NO_3/L) had methemoglobin levels ranging from 1.65% to 2.44% (80 infants). Infants consuming water containing 12-23 mg nitrate-nitrogen/L (51-100 mg NO_3/L) had methemoglobin values of 3.51 (12 infants) and those consuming water containing nitrate at concentrations over 23 mg nitrate-nitrogen/L (100 mg nitrate/L) had methemoglobin levels of 6.59% (4 infants).

Super et al. (1981) examined the association between drinking water nitrate concentration and methemoglobin levels in 486 African infants. The study area was divided into low and high nitrate regions according to whether the drinking water nitrate-nitrogen concentration was <20 mg/L or >20 mg/L. In infants living in the low nitrate region, 13% (38/293) had methemoglobin levels of >3%. In infants living in the high nitrate region, 33% (64/193) had methemoglobin levels of >3%. No further details on individual levels were reported.

Toussaint and Wurkert (1982) studied the methemoglobin levels of 96 infants in Germany as a function of the nitrate concentration in drinking water. A clear dose-response relationship was detected, with mean methemoglobin levels increasing from 1.7% in infants consuming 0-10 mg NO_3/L (0-2.3 mg nitrate-nitrogen/L) to 6.6% in infants consuming >100 mg NO_3/L (>23 mg nitrate-nitrogen/L). Levels tended to be higher in summer months than in the winter, presumably because of greater water intake.

Hegesh and Shiloah (1982) studied the relationship of methemoglobinemia to nitrate or nitrite ingestion in hospitalized infants with diarrhea. Blood nitrate concentrations and methemoglobin levels were measured in 58 infants with diarrhea and 130 controls without gastrointestinal disturbances. Sixty-nine percent of the infants with diarrhea had methemoglobin levels above those for the infants without diarrhea. The authors demonstrated that the infants with diarrhea excreted up to 10 times more nitrate than they ingested, while the controls excreted approximately 2.5 times their nitrate intake. The authors concluded that diarrhea alone can cause infant methemoglobinemia as the result of endogenous nitrate formation.

Johnson et al. (1987) reported the case of a female infant, approximately 2 months old, who died following consumption of well-water containing 150 mg/L nitrate-nitrogen (34.1 mg nitrate-nitrogen/L). The infant's blood methemoglobin concentration was not reported, although she was described as having previous episodes of cyanosis, vomiting and diarrhea. Prior to death her blood was described as chocolate-brown in color.

2. Methemoglobinemia in Older Children and Adults

Craun et al. (1981) conducted an epidemiologic study of 102 children aged 1-8 years in Washington County, Illinois. Sixty-four of the children were from families consuming high-nitrate water (22-111 mg/L nitrate-nitrogen) and 38 children (controls) were from families consuming water containing less than 10 mg/L nitrate-nitrogen. In children aged 1-4 years, ingestion of increasing doses of nitrate in water (from 25 to 500 mg NO_3/24 hours) was found to result in only a slight increase in methemoglobin levels (from 1.00% to 1.36%), a difference which was not statistically significant. Essentially no dose-dependent increase was detected in children aged 5-8 years, whose methemoglobin values averaged around 0.90%-0.95% regardless of nitrate intake from water.

In adults, methemoglobinemia has only been reported following accidental ingestion of large doses of nitrite. Aquanno et al. (1981) described a case involving two laboratory technologists who salted their breakfast with a laboratory salt shaker containing sodium nitrite. Symptoms appearing within 30 minutes included weakness, sweating, nausea, throbbing and a roaring sound in the ears, palpitations, numbness and tingling. Both went to the emergency room for treatment and were found to have cyanosis with methemoglobin levels of 34% and 54%. Treatment with the antidote methylene blue lowered methemoglobin levels to normal in 1 hour.

Walley and Flanagan (1987) reported several cases of methemoglobinemia in humans who had consumed meat containing high levels of nitrite. In one event, a 41-year-old woman and her 18-year-old son consumed meat containing

15,000 ppm nitrite. Blood samples drawn at admission to the hospital revealed 23% methemoglobin in the woman and 7.7% in the son. Their recovery was complete within 12 hours with no treatment other than oxygen. In another case, a 36-year-old man became severely cyanotic and hypoxic 1 hour after consuming meat containing 10,000 ppm nitrite. His blood methemoglobin measured upon arrival at the hospital was 66%. Following treatment with oxygen and methylene blue he recovered. Assuming consumption of about 0.2 kg (0.4 lb) of contaminated meat, these cases probably involved intake of about 2,000-3,000 mg nitrite (about 8-12 mg nitrite-nitrogen/kg).

Kaplan et al. (1990) described 10 cases of severe methemoglobinemia in adults who ingested a meal accidentally salted with $NaNO_2$. One person died and two others were severely affected. Methemoglobin levels in these individuals were 79% and 71%. All patients were tachypneic and cyanotic, and some displayed hypotension, tachycardia, and altered levels of consciousness. No estimate of dose was provided.

B. Teratogenicity and Reproductive Effects

Scragg et al. (1982) and Dorsch et al. (1984) conducted a case-control study in the Mount Gambier region of South Australia to evaluate the relationship between birth defects in children and maternal drinking water parameters. They analyzed 218 case-control pairs identified from birth records available from the years 1951 through 1979, matched by hospital, maternal age, parity and month of delivery. Information about maternal water source was available for each pair. A statistically significant increase in the risk of birth defects (relative risk = 2.8) was reported for women consuming groundwater (5-15 mg/L nitrate) compared to women consuming rainwater (less than 5 mg/L nitrate). The authors emphasized that their results were limited by a number of factors, including possible sampling bias, lack of nitrate exposure data and possible presence of other contaminants in well-water (e.g., chemicals from local wood processing industries or agricultural pesticides). On this basis the authors stated that "it would be premature to interpret our case-control findings exclusively in terms of water nitrate exposure."

Arbuckle et al. (1988) conducted a case-control study to determine the relationship between nitrate concentration in drinking water and differing rates of CNS birth defects in two areas of New Brunswick, Canada. One hundred thirty cases of CNS defects identified from the high- and low-risk areas of New Brunswick for the years 1973-1983 were included in the study. Each case was matched with two randomly selected controls. Compared to a baseline exposure level of 0.1 ppm nitrate in water, exposure to increasing levels of nitrate in well water (1.25-26.0 ppm) was found to be associated with an increased risk of birth defects (relative odds ratio = 2.3), but this was not statistically significant. In contrast, exposure to increasing levels of nitrate in public water (0.36-3.25 ppm) or spring water (0.23-16.7 ppm) was found to result in decreased risk of birth defects (relative odds ratio = 0.39 and 0.0, respectively). These were also not statistically significant.

Aschengrau et al. (1989) performed a case-control study to investigate the relationship between the levels of various chemicals in drinking water and the incidence of spontaneous abortion in women. There were 286 cases and 1,391 controls selected from residents in or close to Boston. Exposure to nitrate in water (0.1-5.5 mg/L) was found to be associated with a decreased risk of abortion (adjusted odds ratio = 0.5), and exposure to nitrite (0.01-0.03 mg/L) was found to have no significant effect on risk (crude odds ratio = 1.1-1.2).

C. Other Noncancer Effects

Inorganic nitrite ion is a direct-acting relaxant of smooth muscle (Nickerson 1975, Moulds et al. 1981). The main effect is on vascular smooth-muscle (especially the venous system) resulting in vasodilation and hypotension. A single oral dose of 180 mg $NaNO_2$ given to an adult male (corresponding to a dose of 0.5 mg nitrite-nitrogen/kg) produced no significant effects as long as the individual was lying down. When raised to a near-vertical position, a number of symptoms characteristic of marked hypotension ensued, including sharply decreased venous pressure, decreased systolic pressure, increased diastolic pressure, increased heart rate, and deep respirations (Weiss et al. 1937). These symptoms disappeared when the patient was returned to a reclining position. Doses of 30-60 mg of $NaNO_2$ are typically employed for therapeutic purposes to treat angina pectoris (Nickerson 1975).

A variety of organic nitrates (e.g., acryl nitrate, nitroglycerin) also produce these same effects, and are usually about 10- to 100-fold more potent than the NO_2^- ion. Inorganic nitrate ion does not produce the effect. The mechanism of vasodilation appears to be by production of S-nitrosothiols which stimulate guanylate cyclase activity in target cells (Herman et al. 1989), perhaps through formation of nitric oxide (NO) (Ignarro 1990).

D. Mutagenicity

No studies were located which investigated the *in vivo* incidence of mutations in any human cell types as a function of nitrate or nitrite exposure. However, it is known that ingestion of nitrate or nitrite can increase the level of various N-nitroso compounds in the body, presumably formed mainly as a result of reactions between nitrite and secondary amines in the stomach (see below). For example, Stemmermann et al. (1981) detected direct-acting mutagens in mucosa removed from the human stomach during surgery. The mutagens were later identified as the N-nitroso derivatives of three drugs (hydroxyzine hydrochloride, diazepam and cimetidine) that had been administered to the patients.

E. Cancer

1. Epidemiological Studies

A number of epidemiological studies have been performed to determine if ingestion of nitrate and/or nitrite is associated with increased risk of cancer (especially stomach cancer) in humans. There are a number of factors which tend to complicate the performance and interpretation of such studies:

- Nitrates and nitrites occur naturally in or are added to many foods, so all humans are exposed through the diet. Intake levels may be quite variable between individuals.

- The mechanism of carcinogenesis is presumed to involve in vivo formations of nitrosamines, so intake of nitrosable substrates (mainly secondary amines) in the diet is also an important parameter that may influence the observed risk levels.

- Antioxidants such as vitamin C and vitamin E are thought to play a protective role, so intake of these materials is also an important variable that may decrease observed risk levels.

- Treatment of food with nitrate or nitrite can sometimes lead to formation of carcinogenic nitrosamines in the food itself before ingestion. This can contribute cancer risk not properly attributable to the nitrate or nitrite intake per se.

- Exposure estimates are often indirect, and are usually available only for the present time. However, cancer risk is more likely a function of past exposure levels.

Some studies have attempted to deal with some of these factors, but many studies have not. Such studies must be interpreted with due caution.

Geographical Correlation Studies

Most studies on cancer risk from nitrate/nitrite are geographical correlation studies, in which average cancer incidence in an area is correlated with average nitrate/nitrite levels in food or water in the same area. This type of study is generally recognized as providing data that is useful in hypothesis formation, but not in establishing causal links. This is because it is not possible to account for multiple risk factors, and no data are collected to show that affected individuals actually had higher exposures to nitrate or nitrite than the unaffected individuals. Several studies of this type are summarized below.

Hill et al. (1973) reported that the death rate from gastrointestinal cancer was higher than expected (SMR = 131 for males and 193 for females) in a town in England where water contained 90 ppm nitrate (20 mg nitrate-nitrogen/L). However, the death rates were not adjusted for the socioeconomic status of the population. In a more careful study of the same population, Davies (1980) adjusted stomach cancer death rates for difference in social class distribution and occupation. After adjustment, the SMR was 97 for men and 123 for women. These values were not significantly different from values in a number of similar towns where nitrate levels in water were not elevated.

Colombia has one of the highest rates of mortality from stomach cancer, especially among persons living at high altitudes in certain rural areas (Haenszel and Correa 1975). Cuello et al. (1976) found that average nitrate levels were higher in wells in the high risk areas (31 mg/L) than in the low risk areas (5.6 mg/L), and that a higher percentage of people in the high risk areas drank water from wells (56%) than in the low risk areas (24%). This evidence of increased nitrate intake in the high risk areas was supported by measurements of urinary nitrite excretion levels which were higher in the high risk than the low risk areas.

Zaldivar and Wetterstrand (1975) studied the correlation between death from gastric cancer and the amount of nitrate fertilizers used in 25 provinces of Chile. A highly significant positive correlation was found between cancer incidence and fertilizer use both for the general population ($P<0.00005$) and for farm workers ($P<0.0001$). The authors interpreted these data to indicate that use of nitrate fertilizers resulted in increased exposure to nitrate, and that this lead to increased risk of gastric cancer by in vivo formation of nitrosamines. A similar association between stomach cancer mortality and use of nitrate fertilizers in Chile was reported by Armijo and Coulson (1975).

Zaldivar and Wetterstrand (1978) studied the relation between death from stomach cancer and nitrate levels in drinking water in urban areas from 25 provinces in Chile. Drinking water levels ranged from 0.0 to 30 ppm nitrate-nitrogen/L. Weak positive correlations were found between nitrate levels and cancer death rate for both men and women, although neither association was statistically significant.

Juhasz et al. (1980) compared the stomach cancer incidence in 230 localities in the County of Szabolcs-Szatmar, Hungary, to the nitrate content of the local drinking water. In general, localities with high nitrate levels (defined as >100 mg NO_3/L) tended to have above average incidence of stomach cancer, but many communities did not fit this pattern. It was concluded that other factors caused stomach cancer in the low-nitrate areas.

Yang (1980) summarized epidemiological studies in several counties in China where incidence rates of esophageal cancer are very high (135/100,000). Numerous factors were compared in high- and low-incidence areas in an effort to identify risk factors. An association was found between increased incidence and a number of dietary factors, including the use of water high in nitrates and nitrites. In the high-risk areas, water was often from shallow ponds that were heavily contaminated with micro-organisms and refuse. Concentrations of nitrate and nitrite averaged 12.6 and 2.6 mg/L, respectively, and the concentration tended to correlate with esophageal cancer incidence rates. Other risk factors included ingestion of nitrosamines in moldy food and pickled vegetables.

Gilli et al. (1984) performed a descriptive epidemiological study in Italy in which the geographical pattern of gastric cancer was compared to geographical variations in nitrate levels in drinking water. They found positive association between nitrate levels and cancer risk, with a relative risk of 13.7 for communities with water above 20 mg/L nitrate (4.5 mg nitrate-nitrogen/L). This study did not control for other important risk factors for gastric cancer (age, diet, alcohol consumption, occupation, socioeconomic status, etc.), so the observed association may have been due to other factors. Also there were large differences in community size, which makes local mortality statistics extremely variable.

Beresford (1985) examined cancer mortality data in 253 urban areas in the United Kingdom, adjusting death rates for variations in socioeconomic status and population size. Drinking water nitrate levels were negatively associated with stomach cancer mortality (that is, stomach cancer death rates were low where nitrate levels were high).

Forman et al. (1985) compared salivary levels of nitrate and nitrite in over 800 healthy male and female volunteers (15-75 years old) from two regions in the United Kingdom recognized as having distinctly different mortality rates for gastric cancer. A written survey was used to evaluate socioeconomic and dietary distinctions between individuals. Mean salivary nitrate and nitrite concentrations in volunteers from 2 areas of the low risk region (208 and 157.3 nmol nitrate/mL; 124 nmol nitrite/mL and 95 nmol nitrite/mL) were significantly greater ($P<0.0001$) than salivary levels of nitrate and nitrite in 2 areas of the high risk region (107 nmol nitrate/mL; 69 and 51 nmol nitrite/mL). Based on this, the estimated total daily intake of both nitrate and nitrite was significantly greater in the low risk group (119.24 mg nitrate; 6.77 mg nitrite) than the high risk group (74.92 mg nitrate; 4.95 mg nitrite).

Takacs (1987) investigated the relationships between incidence of digestive system tumors and the nitrate content of drinking water in Hungary. There was an apparent correlation between incidence and average nitrate concentration across seven districts where water levels ranged from 90 to 230 mg NO_3/L, but a statistical test of trend or significance was not reported.

The high incidence of esophageal cancer in Iran has been studied by a Joint Iran-International Agency for Research on Cancer Study Group (1977). Villages with different rates of esophageal cancer were surveyed for dietary, work and personal habits. Their main foodstuffs were analyzed for volatile nitrosamines, nitrate, nitrite and other compounds. Their drinking water was tested for nitrate and nitrite. The average daily dietary intake of nitrate and nitrite was not significantly different for high- and low-incidence areas, and nitrate and nitrite levels in water were not elevated in areas at high risk for esophageal cancer.

Dutt et al. (1987) measured the nitrate content of a variety of vegetables, fruits and meats consumed by the residents of Singapore. Based on 1982 statistics regarding food imports and production, average daily intake of nitrate was calculated to be 215 mg/day. Two vegetables (Chinese cabbage and kale) were particularly high in nitrate, contributing about 50% of the total. The authors stated that the Malay and Indian subpopulations of Singapore consumed less of these vegetables than the Chinese, and noted that the rate of gastric cancer was lower for these two groups than for the Chinese. The authors noted that this could be a chance association, since there are many other factors that influence the incidence of gastric cancer.

Anquela et al. (1989) investigated the relation between the incidence of gastric cancer and nitrite content of drinking water in nine areas in the province of Soria, Spain. Water concentrations ranged from 0.4 to 11.8 mg nitrite-nitrogen/L. There was a statistically significant ($P<0.01$) positive association between nitrate level and mortality from gastric cancer. The authors noted that highest rates occurred in grain-producing agricultural regions, and that other factors related to agricultural work might also be important.

Cohort Studies

Fraser et al. (1980b, 1982) studied the association between inhalation exposure to nitrate dust and the incidence of various cancers in fertilizer workers in England and Wales. Men (age 35-64) identified from the 1961 and 1971 censuses as "manufacturers of fertilizers or chemicals for pest control" were selected as the study populations. Jobs were coded according to a protocol which estimated the frequency of exposure to nitrate-containing dust as either frequent, infrequent, very infrequent or never. In the 1961 cohort, the ratio of observed to expected deaths (O/E) from all causes was 0.82, consistent with a "healthy worker" effect. Cancer deaths were also lower than expected (O/E=0.85). Only intestinal cancer (O/E=1.33) and bladder cancer (O/E=1.03) had ratios greater than 1.00, but these increases were not statistically significant. In the 1971 cohort, the O/E ratio for all cancers was 1.32. No statistically significant increase in cancer mortality statistics for any of the cancer types were detected, although the O/E ratio for esophageal (2.5), gastric (1.33), rectal (3.33) and bladder (1.41) cancers were greater than 1.00 and were also greater than the ratios seen in the 1961 cohort. An apparent dose-response relationship (not statistically significant) was noted in cancer deaths as a function of exposure duration (O/E = 0.42 for "never" or "infrequent", 1.31 for "infrequent" and 1.69 for "frequent"). No statistically significant differences were observed between groups exposed to different product categories. The authors concluded that this study provided weak evidence of an association between frequency of exposure to nitrate-containing dusts and increased risk of cancer mortality.

Al-Dabbagh et al. (1986) studied mortality patterns in 1,327 male workers in the production of nitrate-based fertilizers in England. Workers employed in such capacity between 1946 and 1981 for at least 1 year were followed up until 1981. Workers were classified into heavy, moderate or low exposure groups based upon their estimated exposure to nitrate. Validation of exposure was accomplished by measuring nitrate and nitrite concentration of saliva in workers (30 per group). Nitrate concentrations in saliva were significantly higher in workers with high (212.3 nmoles/mL) or moderate (206.8 nmoles/mL) exposure to nitrate than in workers with no exposure to nitrates (102.7 nmoles/mL). No significant increase in mortality was observed from cancer or other diseases evaluated in either the entire cohort or the subgroup of workers heavily exposed to nitrates.

Case Control Studies

Haenszel et al. (1973) reported an association between increased risk of stomach cancer in Japanese immigrants to Hawaii and consumption of salt-pickled vegetables (which contain nitrite) along with salted dried fish (which contain nitrosable secondary amines) (Singer and Lijinsky 1976). This suggests that one or both of these dietary components may be a risk factor for stomach cancer, but the data are not adequate to draw firm conclusions.

Risch et al (1985) performed a case-control study on the relationship between dietary history and the risk of stomach cancer in Canada. There were 246 cases and 246 controls, matched by age, sex and area of residence. Dietary history was investigated by questionnaire, and intake of specific dietary constituents (nitrate, nitrite, nitrosamine, vitamins, etc.) was calculated based on U.S. Department of Agriculture data on food composition. Statistically significant associations were noted for intake of nitrite, along with a number of other substances including smoked meats, unsaturated fats, grains, chocolate, eggs, cream desserts and non-refrigerated foods. Intake of fiber, citrus fruits and vitamin C resulted in a significant decrease in risk of stomach cancer. Intake of nitrate also had an odds ratio significantly less than 1.0. The authors concluded that nitrite intake is associated with increased risk of stomach cancer.

Fontham et al. (1986) performed case-control studies to identify dietary risk factors for chronic atrophic gastritis, which is a recognized precursor to gastric cancer (Correa et al. 1976). A total of 93 hospital cases with biopsy-confirmed chronic atrophic gastritis were compared to 186 age- and sex-matched controls in the same hospital. Cases ingested less fruit, vegetables and vitamin C, which was viewed as supporting intragastric nitrosylation as the etiologic mechanism. Stomach pH was higher in cases than controls (a condition favoring bacterial growth), but nitrate and nitrite levels in gastric juice were not higher in cases than controls. This is in contrast to an earlier study by Correa et al. (1979) on nitrite levels in gastric juice of patients with atrophic gastritis from a region in Columbia with a high gastric cancer incidence. In this population, many patients with the disease had increased levels of nitrite in gastric fluid. This was observed only in individuals with stomach pH above 5. For example, three patients with stomach pH values of 7.1, 7.5 and 9.0 had stomach nitrite levels of 193, 136 and 103 ppm, respectively. There was no clear relationship between gastritis, stomach pH and stomach nitrate levels. The authors interpreted these data to indicate that bacterial growth in the stomach above pH 5 could lead to the formation of nitrite, which would increase risk of stomach cancer through formation of nitrosamines.

2. In Vivo Formation of Nitrosamines

Kamiyama et al. (1987) studied the concentrations of nitrate and N-nitrosoamino acids in the urine of inhabitants of two areas of Japan where the risks of stomach cancer were low (33/100,000) and high (101/100,000) respectively. Nitrate levels in urine were lower in the high risk area than the low risk area (116 vs. 140 mg/person/day, $P<0.07$), but levels of N-nitrosothiazolidine-4-carboxylic acid (NTCA) was significantly higher ($P<0.05$). The authors interpreted these data to suggest that the residents of the high-risk area had a greater potential for endogenous nitrosylation, possibly due to the presence of a nitrosylation-inhibiting factor in the low risk area.

Bellander et al. (1988) exposed four healthy adult volunteers to air containing 0.3 mg/m^3 of piperazine. When the volunteers ingested a diet low in nitrate (40-70 mg/d), only one person had measurable levels of nitrosopiperazine in the urine (0.36 μg/32 hr). When ingesting a diet high in nitrites (180-270 mg/day), nitrosopiperazine was detected in three of four samples (mean = 1.2 μg/32hr). This was all found in the urine sample collected 4 hours after the end of piperazine exposure.

Moller et al. (1989) studied the influence of nitrate levels in water on nitrosamine formation in a rural population in Denmark. Participants ingested 500 mg of proline in the evening, and a 12 hour urine sample was collected the next morning and analyzed for N-nitrosoproline. In the whole cohort, there was only a week positive association ($P=0.08$) between nitrate levels in water and nitrosoproline excretion. This was probably due to the large variations between people in the amount of nitrate intake from food. When the cohort was separated into smokers and nonsmokers, nitrosolation was strongly associated with nitrate intake in nonsmokers ($P<0.01$).

Zatonski et al. (1989) studied the concentration of nitrate and N-nitrosoamino acids in the urine of inhabitants of high- and low-risk areas for stomach cancer in Poland. Urinary levels of nitrates were 1.4 higher in the high-risk area ($P<0.01$). Levels of some nitrosoamino acids were not different between the two groups, but levels of N-nitrososarcosine and 3(N-nitroso-N-methylamine)propionic acid were 3-4 fold higher in people from the high risk area ($P<0.02$). When inhabitants were given doses of proline, levels of nitrosoproline increased in the urine of high-risk but not low-risk area inhabitants. The authors concluded that residents of the high-risk area produced higher levels of endogenous nitrosamines than residents of the low-risk area.

F. Summary

The principal adverse health effect associated with exposure of humans to nitrate is methemoglobinemia in infants (age 0-3 months). This group is much more susceptible than older children and adults because the pH of the infant stomach can often be sufficiently high (above pH 4) to permit growth of bacteria that reduce nitrate to nitrite. Many studies have been performed on the levels of nitrate in water that lead to clinically significant methemoglobinemia in infants. These studies have shown that most cases are associated with exposure to water containing 20 mg nitrate-nitrogen/L or higher, and that no significant effects occur if water contains 10 mg nitrate-nitrogen/L or less. In adults, methemoglobinemia has only been reported following accidental ingestion of large quantities of sodium nitrite (usually mistaken for table salt).

Only two studies are available on possible developmental or reproductive effects of nitrate in humans. One study suggested that there might be an increased risk of birth defects associated with ingestion of water containing 5-15 mg nitrate-nitrogen/L, but there were a number of other factors that could also have been responsible for the observation. No significant relation between nitrate levels in water and birth defects was detected in the second study.

Many epidemiological studies have been performed to determine if ingestion of nitrate or nitrite are associated with increased cancer risk. Some of these studies have noted increased cancer risk (especially gastrointestinal cancer) in populations ingesting above average levels of nitrate or nitrite in water or food. However, these studies are not adequate to show that nitrate/nitrite caused the increased risk, since reliable dose-response data are usually lacking, and there are many other risk factors for gastrointestinal cancer that might have been responsible.

VII. MECHANISMS OF TOXICITY

A. Cardiovascular Effect

The acute pharmacological action of nitrite is to relax smooth muscles. The relaxation is nonspecific and affects all smooth muscle irrespective of its innervation or the nature of its responses to adrenergic, cholinergic, or other types of agonists. However, nitrite does not prevent cells from responding to an appropriate stimulus and its effect can be antagonized by any drug that can activate the smooth muscle under consideration. Thus, nitrite is a functional antagonist of norepinephrine, acetylcholine, histamine and many other agents (Nickerson 1975). The mechanism of this effect appears to involve production of S-nitrosothiols which stimulate guanylate cyclase activity in target cells (Herman et al. 1989), perhaps through formation of nitric oxide (Ignarro 1990).

B. Effect on Hemoglobin

Nitrite reacts with hemoglobin in the erythrocytes, oxidizing the iron of hemoglobin to the ferric state. Ferric hemoglobin is called methemoglobin and is unable to transport oxygen (Parks et al. 1981). During formation of metHemoglobin, superoxide ion (O_2^-) is also formed. The superoxide ion is converted by superoxide dismutase to hydrogen peroxide (H_2O_2). This in turn forms a catalase-H_2O_2 complex, for which additional nitrite may act as a substrate and be oxidized to nitrate (Parks et al. 1981).

C. Diuretic Effects

The diuretic effect of very high doses of nitrate in dogs was attributed to enhancement of sodium excretion. This resulted in a net loss of sodium from the body when nitrate was given as the sodium salt (Greene and Hiatt 1954). The rate of excretion of sodium administered with nitrate was much greater than its rate of excretion when given with chloride. In the dog, as much as 40% more sodium was excreted than was injected (as sodium nitrate), bringing about a loss of extracellular fluid.

D. Effects Due to N-Nitrosation

N-Nitrosation of suitable nitrogenous substrates by nitrite in the stomachs of laboratory rodents has been demonstrated by many investigators. Substrates for N-nitrosation by nitrite include secondary amines (which form nitrosamines), N-alkylureas, N-alkylcarbamates and simple N-alkylamides (which form nitrosamides). Tertiary amines can also be nitrosated. Most of these reaction products break down to release nitrosodimethylamine or nitrosodiethylamine (Mirvish 1975).

The amount of nitroso compound produced in the stomach depends partly on the nitrosation kinetics. For nitrosation to occur, nitrite is first converted to HNO_2 (pK_a=3.37). This explains why nitrosation is catalyzed by acid. The reaction rate shows a maximum at pH 3.4. Nitrous acid is then converted to an active nitrosating species, usually nitrous anhydride (N_2O_3) (Mirvish 1975).

Mirvish (1975) concluded the rate of nitrosation is proportional to the concentration of amine and the square of the concentration of nitrite. Therefore, the rate is affected more by an increase in nitrite than by an increase in amine. The rate constants for many secondary amines have been determined. The rate increases as the basicity of the amine decreases (as the pK_a of the amine decreases). Therefore, weakly basic amines react very rapidly.

Thiocyanate increases the rate of nitrosation because the nitrosating species nitrosothiocyanate (ON•NCS) is produced. The optimum pH for nitrosation at high concentration of thiocyanate is 2.0. At this point, nitrite becomes fully protonated. Below pH 2.0, the rate falls because NCS^- becomes protonated. Thiocyanate-catalyzed nitrosation of amines will compete favorably with the N_2O_3 mechanism shown above under three conditions: (1) at pH less than 2.5, (2) at high thiocyanate concentration, or (3) at low nitrite concentration (Mirvish 1975).

Mirvish (1975) concluded that the rate of formation of N-nitroso compounds in the stomach is proportional to the thiocyanate concentration as well as the concentrations of amine and nitrite. Thiocyanate is secreted in human saliva along with nitrate and swallowed into the stomach. Boyland and Walker (1974) showed that at pH 1.5, 1.0 mM thiocyanate increases the rate of the nitrosation reaction about 550 times. Human gastric juice is between pH 1 and 2.

Ascorbic acid in the stomach reacts rapidly with nitrite to reduce it and block the nitrosation reaction. Ascorbic acid is oxidized to dehydroascorbic acid in the process. The blocking is most efficient under anaerobic conditions (Archer et al. 1975).

E. Summary

Methemoglobinemia is the result of a nonenzymic reaction between hemoglobin and nitrite, resulting in oxidation of Fe^{+2} to Fe^{+3}. Nitrate does not cause this reaction unless it is first reduced to nitrite.

Nitrite ion also acts directly on smooth muscle to cause relaxation. The mechanism of this effect is not known precisely, but may involve activation of guanylate cyclase.

Nitrite ion undergoes protonation in the stomach to form nitrous acid, which may react with a variety of secondary and some tertiary amines to form nitrosamines. This reaction is inhibited by ascorbic acid, and can be increased by thiocyanate. Formation of nitrosamines is believed to be the mechanism responsible for the hepatotoxic and carcinogenic effect observed in animals exposed to high doses of nitrite and nitrosable substrates.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals which do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

Quantification of Noncarcinogenic Effects

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), (formerly called the Acceptable Daily Intake (ADI)), is calculated. The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious health effects, even if exposure occurs over a lifetime. The RfD is derived from a No-Observed-Adverse-Effect Level (NOAEL), or Lowest-Observed-Adverse-Effect Level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s). The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{Uncertainty Factor(s)}} = \underline{\quad}\ \text{mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based on professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the Office of Drinking Water (ODW) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS 1977, 1980) as follows:

- An uncertainty factor of 10 is generally used when good chronic, or subchronic human exposure data identifying a NOAEL are available and are supported by good chronic, or subchronic toxicity data in other species.
- An uncertainty factor of 100 is generally used when good chronic toxicity data identifying a NOAEL are available for one or more animal species (and human data are not available), or when good chronic, or subchronic toxicity data identifying a LOAEL in humans are available.
- An uncertainty factor of 1,000 is generally used when limited or incomplete chronic, or subchronic toxicity data are available, or when good chronic, or subchronic toxicity data identify a LOAEL, but not a NOAEL for one or more animal species are available.

The uncertainty factor used for a specific risk assessment is based principally on scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving a RfD, the significance of the adverse health effect and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{(\text{RfD}) \times (\text{Body Weight in kg})}{\text{Drinking Water Volume in L/day}} = \underline{\quad}\ \text{mg/L}$$

where:

Body weight = assumed to be 70 kg for adult

Drinking water volume = assumed to be 2 liters per day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (One-day, Ten-day and Longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using a similar equation to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{(\text{NOAEL or LOAEL}) \times (\text{bw})}{(\text{UF}) \times (\underline{\quad}\ \text{L/day})} = \underline{\quad}\ \text{mg/L}$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. One-day HA for a 10-kg child ingesting 1 L water per day.
2. Ten-day HA for a 10-kg child ingesting 1 L water per day.
3. Longer-term HA for a 10-kg child ingesting 1 L water per day.
4. Longer-term HA for a 70-kg adult ingesting 2 L water per day.

The One-day HA calculated for a 10-kg child assumes a single acute exposure to the chemical and is generally derived from a study of less than 7 days duration. The Ten-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of less than 30-days duration. A Longer-term HA is derived for both the 10-kg child and a 70-kg adult and assumes an exposure period of approximately 7 years (or 10% of an individual's lifetime). A Longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

Quantification of Carcinogenic Effects

The EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

- Group A: Known Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.

- Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

- Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.

- Group D: Not classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.

- Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimate excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually comes from lifetime exposure studies in animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 liters of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure via ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any others. Because each model is based upon differing assumptions, the estimates which were derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose response data usually are available only for high levels of exposure, not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Noncancer Effects

1. Nitrate

Nitrate toxicity is due primarily to its conversion to nitrite, which oxidizes the Fe^{+2} form of iron in hemoglobin to the Fe^{+3} state. This compound (methemoglobin) does not bind oxygen, resulting in reduced oxygen transport from lungs to tissues. Low levels of methemoglobin occur in normal individuals, with typical values usually ranging from 0.5% to 2.0% (Winton et al. 1971, NAS 1981). However, due to the large excess capacity of blood to carry oxygen, levels of methemoglobin up to around 10% are not associated with any significant clinical signs (Walton 1951, Winton et al. 1971, ECETOC 1988). Concentrations above 10% may cause a bluish-color to skin and lips (cyanosis), while values above 25% lead to weakness, rapid pulse and tachypnea (Jones et al. 1973). Death may occur if methemoglobin values exceed 50%-60%.

Conversion of nitrate to nitrite is mostly mediated by bacteria in the alimentary system. Consequently, the risk of methemoglobinemia from ingestion of nitrate depends not only on the dose of nitrate, but also on the number and type of enteric bacteria. In healthy adults, available data suggest that a total of about 5% of a dose of nitrate is reduced to nitrite, mainly by bacteria in the mouth (NAS 1981).

Conversion of nitrate to nitrite may also occur in the stomach if the pH of gastric fluid is sufficiently high (above pH 4) to permit bacterial growth. This may occur in adults with diseases such as achlorhydria or atrophic gastritis, and may also occur in some infants, since the infant gastrointestinal system may have a pH high enough to permit growth of nitrate-reducing bacteria. Reduction of nitrate in the stomach is believed to account for the observation that infants (especially age 0-3 months), are most susceptible to nitrate-induced methemoglobinemia. Risk may be particularly high in infants who are exposed to water that is contaminated with bacteria, for two reasons. First, ingestion of high levels of bacteria in water tends to promote colonization of the gut with organisms that reduce nitrate to nitrite. Second, bacteria in the gut can lead to gastroenteritis and diarrhea, which in turn lead to increased nitrate intake through increased water consumption.

Most cases of methemoglobinemia in humans develop within several hours of ingestion of a dose of nitrate (e.g., Comly 1945, Vigil et al. 1965, Miller 1971, Jones et al. 1973, Kaplan et al. 1990). Thus, methemoglobinemia is most appropriately considered an acute effect. In the absence of repeated doses, recovery is usually complete within several days (e.g., Donahoe 1949), and children continuously exposed show little tendency for cumulative toxicity (e.g., Toussaint and Selenka 1970). In fact, continuous exposure sometimes appears to result in an adaptive response in which methemoglobin levels tend to decrease (Gruener and Toeplitz 1975). On this basis, it is concluded that a value protective for acute exposures will also be protective for longer-term exposures. Therefore, only a single value for nitrate is required, and this will serve as the DWEL as well as the One-day, Ten-Day and Longer-term HA values. Derivation of this value is presented below.

Table VIII-1 Summary of Studies on Methemoglobinemia in Infants

Reference	Route/Medium	Duration	NOAEL*		LOAEL	
			mg/N/kg/d	mg N/L	mg N/kg/d	mg N/L
Cornblath and Hartman 1948	Oral/Water	2-18d	23	140	-	-
Bosch et al. 1950	Oral/Water	Continuous	1.6	10	3.4-8	21-50
Walton 1951	Oral/Water	Continuous	1.6	10	1.8-3.2	11-20
Simon et al. 1964	Oral/Water	Continuous	>3.7	>23	-	-
Toussaint and Selenka 1970	Oral/Formula	10 days	5.4	34	-	-
Winton et al. 1971	Oral/Formula	Continuous	3.6	22	-	-
Shuval and Gruener 1972	Oral/Water	Continuous	2.6	16	-	-
Jones et al. 1973	Oral/Water	1 day	-	-	4.0	25
Gruener and Toeplitz 1975	Oral/Formula	5 days	-	-	3.8	24
Wurkert 1978	Oral/Water	Continuous	>3.7	>23	-	-
Toussaint and Wurkert 1982	Oral/Water	Continuous	>3.7	>23	-	-
Johnson et al. 1987	Oral/Water	-	-	-	5.4	34

*Methemoglobin levels up to 10% are not considered adverse.

RfD for Nitrate

Table VIII-1 lists studies which provide reliable quantitative data on the NOAEL and LOAEL for methemoglobinemia in human infants. Concentrations of methemoglobin less than 10% are not considered adverse, and doses that do not produce levels over 10% are listed as NOAELs.

Based mainly on the studies by Bosch et al. (1950) and Walton (1951), the NOAEL is taken to be 10 mg nitrate-nitrogen/L (1.6 mg nitrate-nitrogen/kg/day). In most studies, the LOAEL is greater than 20 mg nitrate-nitrogen/L (3.2 mg/kg/day), although a small number of cases have been noted at exposure levels between 10 and 20 mg nitrate-nitrogen/L (Walton 1951).

Based on this NOAEL, the RfD for nitrate is calculated as follows:

$$\mathrm{RfD(NO_3)} = \frac{\text{1.6 mg nitrate-nitrogen/kg/day}}{1}$$

$$= \text{1.6 mg nitrate-nitrogen/kg/day}$$

where:

1.6 mg/kg/day = NOAEL (mg nitrate-nitrogen/kg/day), based on weight-of-evidence from studies in human infants

1 = Uncertainty factor. A factor of 10 is often applied to a NOAEL in humans to account for possible variation in sensitivity among individuals. In this case, the NOAEL is based on studies in the most sensitive subpopulation, so no uncertainty factor is required to adjust for this.

DWEL for Nitrate

Based on the RfD of 1.6 mg nitrate-nitrogen/kg/day, the DWEL for nitrate is calculated as follows:

$$\text{DWEL}(NO_3) = \frac{1.6 \text{ mg nitrate-nitrogen/kg/day}}{0.16 \text{ L/kg/day}}$$

$$= 10 \text{ mg nitrate-nitrogen/L}$$

where:

1.6 mg/kg/day = RfD

0.16 L/kg/day = Average fluid intake, based on consumption of 0.64 L/day by a 4-kg infanc (Davidson et al. 1975)

Consideration for Other Possible Noncarcinogenic Effects of Nitrate

There are no convincing data in animals or humans that ingestion of nitrate is associated with any adverse effect other than methemoglobinemia. An epidemiological study in Australia (Dorsch et al. 1984) suggested that there may be an increased risk of birth defects in women consuming groundwater (which contains 5-15 mg/L of NO_3) compared to rainwater (which contains 0-5 mg/L NO_3), but this observation was not taken to constitute proof that nitrate was responsible for the difference in risk. In a similar study in Canada (Arbuckle et al. 1988), no significant association was detected between nitrate intake and risk of birth defects. Also, studies in animals exposed to high levels of nitrate do not indicate any increased incidence of reproductive or teratogenic effects (FDA 1972a,b).

2. Nitrite

Few data exist on nitrite toxicity in humans. Since nitrite is directly toxic (i.e., does not require metabolic conversion by bacteria), toxic effect levels are likely to be less dependent on age than for nitrate. Nevertheless, there do appear to be important differences between infants, adults and animals with respect to the ease of nitrite oxidation of hemoglobin and the rate of methemoglobin reduction back to hemoglobin (Lukens 1987). On this basis, it appears most appropriate to consider the infant the most sensitive subpopulation for nitrite as well as nitrate toxicity. However, there are no reliable quantitative data on the noncancer effects of nitrite ingestion by humans, either for infants or adults. Thus, the RfD for nitrite must be derived by (a) extrapolation of nitrite toxicity data in animals to nitrite toxicity in humans, or (b) by extrapolation of nitrate toxicity data in humans to nitrite toxicity in humans. These options are discussed below.

a. Extrapolation from Nitrite Data in Animals

Table VIII-2 summarizes studies in animals with reliable quantitative data on the toxic effects of nitrite. As in humans (exposed to nitrate), the principal adverse effect associated with exposure is methemoglobinemia. The data are reasonably consistent across studies, and suggest that a dose of up to about 10 mg nitrite-nitrogen/kg/day does not cause any adverse effects, while doses of 20 mg/kg/day or higher can produce methemoglobinemia and other related histological changes. However, a NOAEL of 10 mg nitrite-nitrogen/kg/day is somewhat higher than doses of nitrate-nitrogen known to produce methemoglobinemia in human infants (see Table VIII-1). Since the dose

Table VIII-2 Summary of Studies on the Noncancer Effects of Nitrite in Animals

Reference	Species	Route/ Medium	Duration	Endpoints	NOAEL* mg N/kg/day	LOAEL mg N/kg/day
Shuval and Gruener 1977	Mice	Water	3 weeks	Met Hb	2.6	40
	Rats	Water	6 weeks	Met Hb	54	81
Imaizumi et al. 1980	Rats	Gavage	1 dose	Met Hb	5	10
Druckrey et al. 1963	Rats	Water	70 days	Met Hb	20	-
Shuval and Gruener 1972	Rats	Water	24 months	Met Hb/Growth/Mort.	20	40
Imaizumi et al. 1980	Rats	Water	6 months	Met Hb/Growth/Devel.	50	-
Chow et al. 1980	Rats	Water	16 weeks	Met Hb/Histopathol.	4	-
			14 months	Met Hb/Organ Weight	-	40
Til et al. 1988	Rats	Water	13 weeks	Met Hb/Histopathol.	17	50
Grant and Butler 1989	Rats	Food	115 weeks	Hematology	-	20
Shuval and Gruener 1972	Rats	Water	24 months	Histopathology (lung, blood vessels)	2	20
Inai et al. 1979	Mice	Water	18 months	Histopathology (secondary to methemoglobinemia)	-	41
FDA 1972c,d	Mice	Gavage	GD6-15	Repro/Teratogen.	4.6	-
	Rats		GD6-15		2	-
	Rabbits		GD6-18		4.6	-
	Hamsters		GD6-10		5.3	-

Table VIII-2 (Continued)

Reference	Species	Route/ Medium	Duration	Endpoints	NOAEL* mg N/kg/day	LOAEL mg N/kg/day
Sleight and Atallah 1968	Guinea pigs	Water	100-240d	Repro/Teratogen.	180	192
Druckrey et al. 1963	Rats	Water	3 gen	Repro/Teratogen.	20	-
Shuval and Gruener 1972a,b	Rats	Water	Gest/Lact	Repro/Body Weight	-	54
Globus and Samuel 1978	Mice	Gavage	GD0-18	Repro/Develop.	3.4	-
Hugot et al. 1980	Rat	Food	3 gen	Growth	-	90
Roth et al. 1987	Rats	Water	Gest/Lact	Growth, Neonatal post (lactation)	-	44
				Fetal Hb	10	20
Shiobara 1987	Mice	Gavage	GD6-15	Repro.	8	16
Roth and Smith 1988	Rats	Water	Gest/Lact	Pup growth, Pup histo.	-	40
Shimada 1989	Mice	Water	GD7-18	Repro/Growth/ Teratogenicity	49	-

*The NOAEL for methemoglobinemia is taken to be 10%.

of nitrite to the infant cannot exceed the dose of nitrate (and is presumably somewhat less), these data indicate that animals are not as sensitive to nitrite as human infants. Therefore, extrapolation of nitrite toxicity data from animals to humans does not appear to be prudent.

b. Extrapolation from Nitrate Toxicity Data in Humans

The dose of nitrite formed in the body from an ingested dose of nitrate can be calculated as follows:

Dose of Nitrite = Dose of Nitrate x Percent Conversion

Using this approach, the NOAEL for nitrite in humans may be estimated from the NOAEL for nitrate, if percent conversion is known.

The percent conversion for older children and adults is estimated to be about 5% (NAS 1981), but a reliable NOAEL for methemoglobinemia in this group is not available. Even if the NOAEL for nitrate were known, an estimate of the NOAEL for nitrite in this group might not be protective for infants, due to the more rapid oxidation and slower reduction of hemoglobin in infants (Lukens 1987).

Ideally, the NOAEL for nitrite should be based on the NOAEL for nitrate in infants times the percent conversion in infants. While the NOAEL is known with reasonable accuracy (i.e., 10 mg nitrate-nitrogen/L), there is no reliable information on the percent of nitrate conversion to nitrite in infants.

In the absence of data, the NOAEL for nitrite may be estimated by assuming a conversion rate in infants. It is important to note that, in this case, it is not conservative to assume 100% conversion of nitrate to nitrite; rather, the smaller the assumed conversion, the lower the estimated dose of nitrite. For example, based on a NOAEL of 10 mg/L for nitrate-nitrogen, the NOAEL for nitrite would be 10 mg/L if 100% conversion is assumed, but only 1 mg/L if 10% conversion is assumed. Based on the data which indicate that conversion of nitrate to nitrite is at least 5% in adults (NAS 1981), and knowing that nitrate reduction in infants is significantly greater than in adults due to the presence of gastrointestinal bacteria, the conversion rate is estimated to be at least 10% in infants. This is likely to be substantially lower than the actual conversion rate in individuals who are susceptible to methemoglobinemia, since they generally have a high gastrointestinal content of bacteria that reduce nitrate to nitrite. Employing this percent conversion, the RfD and DWEL for nitrite may be calculated as follows:

RfD for Nitrite

$$\text{RfD}(NO_2) = 1.6 \text{ mg } NO_3\text{-nitrogen/kg/day} \times \frac{0.1 \text{ mg } NO_2\text{-nitrogen}}{\text{mg } NO_3\text{-nitrogen}}$$

$$= 0.16 \text{ mg nitrite-nitrogen/kg/day}$$

where:

1.6 mg/kg/day = RfD for nitrate in human infants

0.1 = Conservative estimate of fraction of nitrate reduced to nitrite kg infants.

DWEL for Nitrite

$$\text{DWEL}(NO_2) = \frac{\text{0.16 mg nitrite-nitrogen/kg/day}}{\text{0.16 L/kg/day}}$$

$$= \text{1.0 mg nitrite-nitrogen/L}$$

where:

0.16 mg/kg/day = RfD for nitrite

0.16 L/kg/day = Average fluid intake, based on 0.64 L/day by a 4-kg infant (Davidson et al. 1975)

Consideration of Other Possible Noncancer Effects of Nitrite

Studies in animals provide little evidence of noncancer effects other than methemoglobinemia from ingestion of nitrite. No evidence of reproductive or teratogenic effects has been noted in several studies, except at doses that produce severe iron deficiency in the dam's milk (Roth et al. 1987). A few reports identify histological changes in the lung (Shuval and Gruener 1972), coronary arteries (Shuval and Gruener 1977), spleen (Fritsch et al. 1980) and adrenal cortex (Til et al. 1988), but none of these effects appear to result in any functional impairments at doses that produce methemoglobinemia.

B. Cancer

1. Weight-of-Evidence Evaluation

Most studies of nitrate or nitrite ingestion by humans or animals have yielded negative or equivocal evidence of carcinogenicity. A number of studies have shown that animals fed nitrite along with very high doses of nitrosable substrates have increased incidence of tumors. The USEPA is currently evaluating these data, but a weight-of-evidence classification has not yet been assigned for either nitrate or nitrite.

2. Quantification of Cancer Risk

The USEPA has not performed quantitative cancer risk calculations for either nitrate or nitrite.

C. Existing Guidelines and Standards

The World Health Organization recommends a limit of 10 mg/L nitrate-nitrogen and less than 1 mg/L nitrite-nitrogen in drinking water (WHO 1984).

D. Susceptible Subpopulations

Infants are at greatest risk of developing methemoglobinemia from nitrate exposure. This increased susceptibility is related to at least four factors (Phillips 1971). First, fetal hemoglobin is more readily oxidized than hemoglobin in adults. At birth, between 60% and 80% of the hemoglobin remains in the immature form of the molecule, hemoglobin F. This decreases to approximately 30% by the third month of life (Pisciotta et al. 1959). Second, the infant has a transitory physiologic deficiency of methemoglobin reductase or its cofactor, NADH (reduced nicotinamide adenine dinucleotide), which are necessary to maintain iron in its reduced state (Ross and Desforges 1959). Third, on a weight basis, infants consume over five times as much water (the most important source of nitrate in the etiology of methemoglobinemia) as adults (Burden 1961). Finally, relative achlorhydria in the very young favors overgrowth of nitrate-reducing bacteria in the stomach (Walton 1951).

Individuals with altered physiological states or with either hereditary or acquired disease may also be predisposed to the development of nitrite- or nitrate-induced methemoglobinemia. These include adults with reduced gastric acidity (including those being treated for peptic ulcer or individuals with chronic gastritis or pernicious anemia) and individuals with a rare, hereditary lack of NADH or methemoglobin reductase activity in their red blood cells (Scott 1960). This hereditary enzyme deficiency seems also to account for the somewhat increased incidence of methemoglobinemia among Alaskan Eskimos and Indians (Scott and Hoskins 1958).

Pregnant women may also be more susceptible than average adults to nitrate-induced methemoglobinemia. Skrivan (1971) reported that methemoglobin levels tended to increase during pregnancy, reaching a maximum of about 9%-10% between 30-35 weeks of gestation. The basis for the increase was not identified, but might be due to a reduced rate of methemoglobin reduction. No evidence of cyanosis was observed in the pregnant women, and no significant differences in methemoglobin levels were detected between women with normal pregnancies and those with premature deliveries or abortions. Although these observations do not indicate that the elevation of methemoglobin during pregnancy is a risk factor for abnormal births, pregnant women may be more likely to experience increased methemoglobin levels following nitrate/nitrite exposure.

E. Summary

Epidemiological studies and case reports from the United States and elsewhere indicate that clinically significant methemoglobinemia does not occur in infants who ingest water containing 10 mg nitrate/nitrogen/L or less. This concentration corresponds to an average daily intake of 1.6 mg nitrate-nitrogen/kg/day by an infant, and this is taken to be the Reference Dose (RfD) for nitrate for an infant. This value is also protective for older children and adults, since infants are the most sensitive subpopulation.

Data are not available to identify the NOAEL for nitrite in infants. Based on the assumption that infants convert at least 10% of ingested nitrate to nitrite, the NOAEL is estimated to be at least 1 mg nitrite-nitrogen/L. Based on this, the RfD for nitrite is 0.16 mg nitrite-nitrogen/kg/day for an infant. This value is also protective for older children and adults, since they are somewhat less susceptible to nitrite-induced methemoglobinemia than infants.

The USEPA is currently evaluating the evidence that ingestion of nitrate or nitrite may be associated with increased risk of cancer. Cancer weight-of-evidence categories have not yet been assigned.

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APPENDIX 1

EPA RESPONSES TO REVIEW COMMENTS PROVIDED BY THE SCIENCE ADVISORY BOARD

The Drinking Water Subcommittee (now a full committee) of the USEPA Science Advisory Board (SAB) performed a thorough review of the Final Draft Criteria Document for Nitrate/Nitrite (dated May, 1987). Review of comments from the SAB were forwarded to the administrator (Hon. Lee M. Thomas) by Richard Griesemer and Norton Nelson, chairmen of the SAB, in a letter dated May 11, 1987. This appendix summarizes the comments by the SAB, and provides EPA's responses.

Comment:

The documentation and its presentation are incomplete and confusing, thus creating difficulties in reaching conclusions on matters of scientific interpretation. For example, the observations of Walton are used as a basis for determining acceptable levels for a ten-day health advisory. The Subcommittee is particularly concerned about the weight given the Walton study. The document describes the study in the summary with little or no interpretation of the underlying study design, but its importance merits an expert review by an epidemiologist. The study needs to be fully described in the body of the document along with conclusions as to its limitations and relative importance. Further, the same critical approach should be followed for all studies that are crucial to the development of proposed standards.

Response:

The organization of the text has been revised to facilitate the task of data interpretation. The description of the Walton study has been expanded to provide greater detail on the nature of the study and its limitations. Similar detail has been added to other key studies. The Walton study is considered to be only one of many studies in humans which support the proposed standard for nitrate, and the text has been revised to make this clear.

Comment:

Public health standards setting involves the examination and selection of appropriate margins of safety. The Safe Drinking Water Act and its later Amendments specify that EPA address this issue. It is especially critical here since the document cites the National Academy of Sciences report (Drinking Water and Health, 1977) that remarks on the narrow margin of safety for nitrates. In the Criteria Document for Nitrate and Nitrite, the Agency selects a margin of safety that excludes, for all practical purposes, protection of sensitive members of the population, namely, infants with gastrointestinal disease. The staff should clarify it technical rationale for developing a margin of safety, as well as the need to include or exclude particular subgroups of the population, for example, those individuals who are genetically disposed to only slowly reduce the methemoglobin.

Response:

EPA believes that the standard of 10 mg/L contains an adequate margin of safety to protect sensitive members of the population, namely, infants with gastrointestinal disease. This conclusion is supported by numerous analyses of the scientific literature. For example, both the World Health Organization (Guidelines for Drinking Water and Other Supporting Information, 1984, WHO, Geneva, pg 128-134) and the Canadian Department of Health and Welfare (Guidelines for Canadian Drinking Water Quality, Supporting Documentation, 1978, pg. 419-431, Minister of Supplies and Services, Canada, 1980) concluded that infant methemoglobinemia has not been reported where drinking water contains less than 10 mg/L nitrate.

Comment:

The Office of Drinking Water should set a single public health standard for the contribution from both nitrate and nitrite.

Response:

EPA agrees and proposes a combined nitrate/nitrite MCLG of 10 mg/L.

Comment:

The subcommittee notes that the document limits the consideration of exposure to oral ingestion of drinking water. The document should attempt to place into perspective the contributions of drinking water to total human health exposure by age group (particularly the endogenous sources); at present, there is little opportunity to determine to what extent Agency actions for drinking water will provide overall public health protection. The Subcommittee recommends that the Office of Drinking Water also expand the chapter on alternate pathways and sources of exposure to provide a more comprehensive analysis of relative exposure, and that the section on the quantification of toxicological effects examine in detail the contribution of nitrate and nitrite in drinking water to the risk of disease in various populations.

Response:

EPA has analyzed nitrate/nitrite exposure through various pathways. That analysis is described in a document entitled "Estimated National Occurrence and Exposure to Nitrate/Nitrite in Public Drinking Water Supplies". Available information indicates that adults who consume nitrate at the proposed standard of 10 mg/L would receive approximately 50% of their nitrate from drinking water and 50% from the diet.

Comment:

A data gap exists for reproductive and developmental effects that the document should cite. If reproductive and developmental toxicity data are not available from either reliable human epidemiologic studies, from concretely relatable human experience, or from valid state-of-the-art animal studies, this fact itself must be stated. Furthermore, when developmental toxicity data are available from animal studies, then both the NOEL and the magnitude of the most likely margin of safety between that animal NOEL and probable human exposure needs to be stated. A second point is the relationship of the vulnerability of the conceptus and the mother to the agent. If the agent in question produces developmental toxicity only at, or very near to, maternally toxic dose levels in an animal study, this needs to be stated as does the more hazardous situation wherein an agent produces adverse effects on the conceptus in the absence of any maternal homeostatic effect.

Response:

The revised criteria document contains summaries of numerous reproductive studies of nitrate and nitrite in animals which indicate there is low risk of teratological or developmental effects except at exposure levels that produce marked maternal methemoglobinemia. The EPA believes these data are adequate to show that reproductive effects are not the most sensitive endpoint of nitrate/nitrite toxicity. The few studies that have been reported in humans are too limited to draw firm conclusions. The text has been revised to expand on this important topic.

Comment:

There is a carcinogenic potential of these inorganic ions in chemical combination with naturally occurring substances. The Subcommittee recommends that the Office of Drinking Water present a conclusion on the current knowledge about the potential for impacts of nitrosated materials from nitrate and nitrite in drinking water. The Criteria Document may require further revision if new information arises about this subject. Specifically, it is desirable to consider the case of splenic sarcomas in male rats which appear to be related to compounds that produce methemoglobinemia. The Subcommittee also recommends that the nitrosamine issue (endogenous formation) could warrant a separate EPA position paper as some nitrosamines are likely to be human carcinogens.

Response:

The Criteria Document has been revised to provide detailed review of studies on the carcinogenicity of nitrate alone, nitrite alone, and nitrite plus nitrosable substrates, as well as the potential to form nitrosamines *in vivo*. The relationship between splenic tumors and methemoglobinemia has been investigated, and there does not appear to be a strong link. Bus and Popp (1987) noted that the only compounds which have been found to produce splenic toxicity and carcinogenicity are structurally related to aniline, suggesting that the key chemical requirement is an aromatic amine. The mechanism of toxicity/carcinogenicity by such compounds is not known, but is suspected to involve oxidation of phenylhydroxylamine (a metabolite of aniline produced in the liver) to nitrosobenzene in the erythrocyte, a reaction that results in methemoglobin formation. However, it is the ability to form nitrosobenzene, not methemoglobin, that appears to be responsible for damage to the erythrocyte and subsequent toxicity/carcinogenicity to the spleen. It should be noted, that except for methemoglobin, nitrate/nitrite exposure usually produces only mild effects on erythrocytes, and there is no evidence that nitrate/nitrite result in nitrosobenzene formation or splenic toxicity.